A Review of Naproxen/Aspirin Pharmacodynamic Interaction Data Including the Results of the Kontakt Study

Joint Meeting of the Arthritis Advisory Committee (AAC) and the Drug Safety and Risk Management Advisory Committee (DSaRM)

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<th>Abbreviation</th>
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<tr>
<td>AA</td>
<td>Arachidonic acid</td>
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<tr>
<td>ASA</td>
<td>Aspirin</td>
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<tr>
<td>BID</td>
<td>Twice daily</td>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
<td>COX-1</td>
<td>Cyclooxygenase-1</td>
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<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>CV</td>
<td>Cardiovascular</td>
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<tr>
<td>CT</td>
<td>Closure time</td>
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<tr>
<td>EC</td>
<td>Enteric-coated</td>
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<tr>
<td>IR</td>
<td>Immediate-release</td>
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<tr>
<td>MACE</td>
<td>Major adverse cardiovascular events</td>
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<tr>
<td>nNSAIDs</td>
<td>Non-selective non-steroidal anti-inflammatory drugs</td>
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<tr>
<td>NSAIDs</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>OTC</td>
<td>Over-the-counter</td>
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<tr>
<td>PGH</td>
<td>Prostaglandin endoperoxide H</td>
</tr>
<tr>
<td>PGG2</td>
<td>Prostaglandin G2</td>
</tr>
<tr>
<td>PGH2</td>
<td>Prostaglandin H2</td>
</tr>
<tr>
<td>PRP</td>
<td>Platelet-rich plasma</td>
</tr>
<tr>
<td>QD</td>
<td>Daily</td>
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<tr>
<td>QID</td>
<td>Four times a day</td>
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<tr>
<td>TID</td>
<td>Three times a day</td>
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<tr>
<td>Tx</td>
<td>Thromboxane</td>
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<tr>
<td>TxA2</td>
<td>Thromboxane A2</td>
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<td>TxB2</td>
<td>Thromboxane B2</td>
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EXECUTIVE SUMMARY

Aspirin is the bedrock of antiplatelet treatment strategies to prevent recurrent ischemic stroke and myocardial infarction. The antiplatelet property of aspirin is attributed primarily to irreversible acetylation of the platelet cyclooxygenase (COX)-1 enzyme, and subsequent inhibition of thromboxane A2 (TxA2) generation and TxA2-induced platelet aggregation. TxA2 is highly unstable and rapidly hydrolyzed to the physiologically stable inactive metabolite, thromboxane B2 (TxB2). Therefore, serum TxB2 measurement is widely used as a surrogate for platelet COX-1 activity, and to indicate TxA2 generation and COX-1 specific effects of aspirin.

A large number of patients treated with aspirin, particularly older patients, also use over-the-counter (OTC) non-steroidal anti-inflammatory drugs (NSAIDs) for pain relief. Low dose naproxen sodium is a commonly-used and widely available OTC NSAID. When taken as directed, it is a safe and effective OTC pain reliever to temporarily relieve minor aches and pains, including that of arthritis, headache, backache, muscular aches, toothache and menstrual cramps, since its introduction as a self-care medication in 1994. In over 20 years of safety data monitoring and availability as a non-prescription pain reliever, Bayer’s pharmacovigilance data indicate no signal or trend with regard to the occurrence of cardiovascular (CV) thrombotic and overall CV events. In addition, naproxen has demonstrated low CV thrombotic risk across a large, existing body of evidence from randomized, controlled clinical trials, observational studies and 40+ years of real-world use of prescription and non-prescription doses.

In contrast to aspirin, which inhibits platelet COX-1 for the lifetime of the platelet, several non-selective NSAIDs including naproxen sodium, bind reversibly to COX-1 and thereby provide a temporary antiplatelet effect. Despite this difference, OTC 220 mg immediate-release (IR) naproxen sodium administered two or three times per day can reduce serum TxB2 to a similar degree as low-dose (81 mg/day) aspirin. However, since naproxen binds to COX-1, questions have arisen regarding a potential pharmacodynamic interaction between naproxen and low-dose aspirin that may depend on the timing of administration of each drug.

In discussions with FDA, Bayer agreed to the design and conduct of a drug interaction study using immediate release aspirin 81 mg and the lowest OTC dose and dosing regimen of naproxen sodium to further investigate the possibility of a drug interaction between aspirin and naproxen. The aim of the study (Kontakt Study) was to investigate whether concurrent administration of naproxen sodium 220 mg once (QD) or twice (BID) daily results in a pharmacodynamic interaction when concurrently administered with a low-dose (81 mg/day) immediate release aspirin regimen. The interval of intake between naproxen and aspirin dosing was also examined to determine whether timing influenced a potential pharmacodynamic interaction.

One challenge with designing such studies is the lack of an established laboratory surrogate marker associated with cardioprotection. Currently, there is no standard that defines the optimal percent inhibition of serum TxB2 for prevention of secondary cardiovascular events. Importantly, there is no understanding of the relation of serum TxB2 inhibition to the occurrence of clinical events. Whether the relation is linear, exponential or stepwise is unknown. The threshold for defining a pharmacodynamic interaction in the Kontakt study was the lower bound of the one-sided 95% CI for the inhibition of serum TxB2 less than 95%. This definition was based on the demonstration that this high level of ex vivo inhibition is required for in vivo inhibition of thromboxane A2 generation as indicated by measurement of urinary 11-dehydro-
thromboxane B2 (11-dh TxB2). However, there is variability in the peer-reviewed literature on the appropriate degree or threshold for serum TxB2 inhibition that is associated with adequate platelet inhibition. Some studies recognized ≥90% TxB2 inhibition as the test threshold for adequate platelet inhibition that marks an optimal antiplatelet effect of aspirin.

The Kontakt study was rigorously designed to maximize the potential for observing an interaction and the pharmacodynamic threshold for declaring interaction was set high at 95%.

Results of the Kontakt study show:

- Evidence of a pharmacodynamic interaction was not observed during the first day of concurrent treatment. These findings suggest that in individuals on a low dose aspirin regimen, use of a single dose of naproxen sodium would not reduce TxB2 inhibition below the 95% threshold.

- After 10 days of concurrent treatment, irrespective of the timing and dose of naproxen in relation to aspirin dosing, a pharmacodynamic interaction was observed in all of the concurrent treatment groups. The number of days of concurrent dosing before TxB2 inhibition falls below the 95% threshold cannot be determined from this study as no samples were collected on Days 2-9 of concurrent dosing.

- The degree of pharmacodynamic interaction appears to be influenced by the timing of aspirin and naproxen dosing when used concurrently over multiple days. Subjects assigned to low dose aspirin 30 minutes before once daily naproxen sodium 220 mg achieved >94% thromboxane inhibition (lower bound of the 95% one-sided confidence interval), narrowly falling short of the 95% threshold.

- After 10 days of concurrent use of low dose aspirin and naproxen sodium, evidence of a pharmacodynamic interaction persisted for fewer than 3 days after discontinuing naproxen sodium among subjects administered 81 mg immediate release aspirin plus once daily naproxen sodium 220 mg and for at least 3 days among subjects treated with low dose aspirin plus twice daily naproxen sodium 220 mg.

- In the control group (aspirin alone), the lower bound for serum TxB2 inhibition was >98% at all time points.

The clinical relevance of this pharmacodynamic interaction remains unknown. Results of PRECISION released in Nov 2016 indicated celecoxib was non-inferior to ibuprofen and naproxen in terms of CV risk. Published data on concurrent intake of prescription doses of naproxen and aspirin generally do not show an increase in CV events. However, no large, randomized, well-controlled clinical outcome trials designed specifically to address this endpoint have been conducted.
1. **INTRODUCTION**

Pain is common and often co-exists with cardiovascular disease. Co-existence of pain and cardiovascular disease tends to increase with age. Consequently, among the aging population in the US and other countries many people will take both nonsteroidal anti-inflammatory drugs (NSAIDs) for the relief of pain and low dose aspirin for cardiovascular event prevention. In the US, it has been estimated that in 2010, approximately 43 million adults (19.0%) were regular aspirin users (at least three times per week for more than 3 months), and more than 29 million adults (12.1%) were regular users of prescription or over-the-counter (OTC) NSAIDs. It was further estimated that 16.0% of all those taking either an NSAID or aspirin used both simultaneously\(^1\). Another estimate suggested that concurrent use of low dose aspirin is present in more than 20% of all patients taking either NSAIDs or COX-2 inhibitors.\(^2\)

This high level of concurrent use is important because a potential interaction between aspirin and some NSAIDs may compromise the cardiovascular prevention effects of aspirin.\(^3\) A landmark study by Catella-Lawson et al.\(^4\) showed that a clinical OTC dosing regimen of ibuprofen may competitively inhibit the sustained inhibitory effect on platelets that underlies the cardioprotective property of aspirin. In 2006, the Food and Drug Administration issued a notification to healthcare professionals about the interaction of ibuprofen with the antiplatelet effects of low dose aspirin.\(^5\) It was uncertain if the interaction seen with ibuprofen also applied to other NSAIDs, all of which have their own unique pharmacokinetic and pharmacologic properties. Subsequently, Bayer sponsored two pharmacodynamic studies that examined the platelet inhibitory effects of OTC naproxen sodium \(^6\) and the interaction of maximum OTC naproxen sodium with low dose aspirin.\(^7\) FDA informed Bayer that the results of these studies and results of published literature do not rule out the possibility of a drug interaction between aspirin and naproxen sodium. In consultation with FDA, Bayer agreed to the design and conduct of a drug interaction study using immediate release aspirin and the lowest OTC dose and dosing regimen of naproxen sodium to further investigate the possibility of a drug interaction between aspirin and naproxen.

2. **ANTIPLATELET ACTIVITY OF LOW DOSE ASPIRIN**

2.1 **Aspirin Pharmacology**

In the early 1970s, the inhibition of prostaglandin biosynthesis was proposed as aspirin's mechanism of action.\(^8\) Since the 1990s, it has been known that NSAIDs impart analgesic and anti-inflammatory effects by blockade of cyclooxygenase (COX), or prostaglandin H-synthase. COX exists in two isoforms: COX-1, which is constitutively expressed in most cells, and COX-2, which is induced by proinflammatory stimuli (cytokines, growth factor, shear) in endothelial cells, monocytes/macrophages, tumor cells, and plaque-associated cells. In general, COX-2 expression is minimal under basal conditions. COX mediates the production of eicosanoids that serve multiple roles in normal homeostasis and disease. Prostanoid biosynthesis is initiated via release of arachidonic acid (AA) from the cell membrane by lipases, primarily phospholipase A2, and subsequent conversion by prostaglandin (PG) H-synthase to PGH2. PGH synthase has both COX activity that converts AA to PGG2 and peroxidase activity that converts PGG2 to PGH2.
The role of AA metabolites generated by COX in the homeostasis of vascular tone and thrombosis has been well described. PGH2 is metabolized by tissue-specific isomerases such as thromboxane synthase in platelets to TxA2; PGI synthase in endothelium, vascular smooth muscle cells, and renal cells to PGI2; and PGE synthase in gastric mucosa and renal cells to PGE2. In addition, leukocytes, vascular smooth muscle cells, endothelial cells, and platelets express PGE synthase and, as a result, are all capable of generating the inflammatory prostanoid, PGE2.\textsuperscript{9,10,11,12}

2.2 Platelets and Thromboxane
Platelets play an important role in hemostasis and thrombosis. Upon activation, the platelet response is amplified by the activation of phospholipase A2 which produces AA from membrane phospholipids.\textsuperscript{10} Arachidonic acid is then metabolized in a series of enzymatic reactions involving COX-1 and thromboxane synthase to produce TxA2. Thromboxane A2 induces platelet aggregation and vasoconstriction (prothrombotic effects). Thromboxane synthase is platelet specific and therefore, platelets are the major sources of TxA2.

2.3 Antiplatelet Activity of Aspirin
The cardioprotective antiplatelet activity of a low dose aspirin regimen is well established (Professional Labeling, 21 CFR 343.80). Aspirin therapy provides inhibition of thromboxane mediated platelet activity via an irreversible inhibition of the COX-1 enzyme.

Aspirin’s antiplatelet activity is mediated through its ability to permanently acetylate a serine residue (serine 530) near the active site of the platelet COX-1 enzyme. This causes an irreversible inhibition of the platelet COX-1 enzyme, resulting in an attenuation of TxA2 formation, and TxA2-induced platelet aggregation.\textsuperscript{13} As platelets are anucleate and cannot synthesize new proteins, the action of aspirin on platelet cyclooxygenase is sustained for the life of the platelet (7-10 days).\textsuperscript{13,14,15,16} Thus, repeated daily doses of aspirin produce a cumulative effect on platelet function, by irreversibly inhibiting newly formed platelets on an ongoing basis. Platelet COX-1 inhibition serves as the basis of the cardioprotective effects of aspirin and other NSAIDs.

This antiplatelet effect is associated with prolongation of the bleeding time and inhibition of TxA2-dependent platelet aggregation.\textsuperscript{15} Since TxA2 is highly labile, thromboxane B2 (TxB2), its more stable metabolite, is used as a surrogate for assessing platelet inhibition.\textsuperscript{17,18,19} Ex vivo TxB2 inhibition \( \geq 95\% \) has been suggested as a threshold to delineate adequate antiplatelet activity based on previous demonstration that this level of inhibition ex vivo is required for in vivo inhibition of thromboxane A2 production determined by urinary 11-dh TxB2.\textsuperscript{20} Several studies have demonstrated the efficacy of low dose oral aspirin (81 to 100 mg per day) in preventing platelet thromboxane production. It is well established that 100 mg of aspirin per day is sufficient to significantly reduce thromboxane production.\textsuperscript{14} A single 100 mg aspirin dose reduced serum TxB2 by 98% during the first hour.\textsuperscript{21,22,23,24} Similarly, in a longer-term study, after seven daily doses of enteric-coated, low dose (81 mg) aspirin, the mean percentage inhibition from baseline of ex vivo generated serum TxB2 was 97.4%, compared with a 7.8% increase after placebo treatment.\textsuperscript{25} Other clinical trials have shown that 6 days of dosing with
immediate release (IR) aspirin 81 mg produces greater than 98% inhibition of thromboxane B2. \(^4_{,26}\)

Aspirin (acetylsalicylic acid) is only transiently exposed in the circulation, with a half-life of approximately 15-20 minutes (Needs, 1985). Following oral administration, aspirin is rapidly deacetylated to salicylic acid, an active metabolite. Once deacetylation occurs, however, the ability to inhibit platelet COX-1 activity is lost.\(^27\) Consequently, a narrow time window exists for aspirin to acetylate platelet COX-1 and inhibit thromboxane production. Despite this, daily exposure to even low doses of aspirin produce a cumulative and nearly complete inhibition of platelet-derived thromboxane production.\(^{18}\)

3. NSAID ASPIRIN INTERACTIONS

3.1 Antiplatelet Effects of NSAIDs

NSAIDs are classified based on their relative selectivity in inhibiting COX-1 and COX-2. Nonselective NSAIDs inhibit both COX-1 and COX-2. Coxibs are NSAIDs that are COX-2 specific. Non-selective NSAIDs (such as ibuprofen and naproxen) are capable of inhibiting platelet activity. The antiplatelet effects of ibuprofen, naproxen and other non-selective nonsteroidal anti-inflammatory drugs (NSAIDs) are mediated through COX-1 inhibition, blocking formation of TxA2, and preventing thromboxane-dependent platelet aggregation. Unlike aspirin, however, ibuprofen, naproxen and other NSAIDs bind reversibly to the COX enzyme. Due to the transient nature of this binding, NSAID molecules eventually disengage the COX enzyme, which if left unoccupied, can once again contribute to the production of thromboxane. The extent and duration of NSAID binding to platelet COX-1 and thus thromboxane inhibition varies based on the affinity for the COX-1 enzyme, as well as the pharmacokinetic profile of the individual agent.\(^{16,28,29}\)

3.2 Antiplatelet Activity of Naproxen

The antiplatelet effects of naproxen have been examined in studies involving a limited number of subjects. For example, Hinz et al.\(^{30}\) assessed the impact of naproxen sodium 220 mg twice daily for 7 days on COX-1 activity (assessed via serum thromboxane B2) in four healthy volunteers. Blood samples were obtained pre-dose, at specified time points after the first dose on Day 1, and 12 hours after the evening dose on Days 2-5 and 8. Recovery was assessed up to 36 hours after the last dose. Results for single dose administration of 220 mg of naproxen sodium (Day 1) demonstrated 94% TxB2 inhibition at the first hour following dosing, with modest recovery observed in the time points that followed. TxB2 was inhibited approximately 80% 12 hours post-dose on Day 1 and approximately 93% inhibited 12 hours post-dose on Day 3 (Figure 1).
In another small study, Capone et al. studied thromboxane inhibition with multiple-day OTC doses of naproxen sodium alone. Six healthy volunteers were given either 220 mg naproxen sodium twice daily, 440 mg naproxen sodium twice daily or aspirin 100 mg daily for 6 days and then crossed-over with a 14-day washout period in between each treatment period. Serum TxB2 levels were assessed prior to dosing and on the sixth day of each naproxen treatment period at 2, 5, 8, 12, and 24 hours following the last dose. Assessments were made at 1 and 24 hours following the last dose in the aspirin only arm of the study. For both 220 mg and 440 mg, Capone et al. reported rapid and relatively complete inhibition of thromboxane at 2 hours after the last dose on Day 6 (95.9±5.1% and 99.2±0.4%, respectively). A modest decline in TxB2 inhibition was reported for the 220 mg dose, at 5, 8, and 12 hours post-dose on Day 6 as shown in Figure 2. At 24 hours post-dose, thromboxane inhibition was reported to be 69±19.9%. The
data related to the 440 mg dose was similar to that of 220 mg; however significant thromboxane inhibition (greater than 90%) was sustained through 12 hours after the last dose on Day 6. At 24 hours post, thromboxane inhibition was reported to be 85±5.1%. As illustrated in Figure 2, AA-induced platelet aggregation remained consistently inhibited up to 8 and 12 hours after the last dose on Day 6 for 220 mg and 440 mg naproxen sodium respectively.
Figure 2 Capone Study 2007. Comparison of degree and duration of steady-state inhibition of COX-1 activity and platelet function ex vivo by naproxen sodium at 220 and 440 mg b.i.d. or low dose aspirin for 6 days.

Comparison of degree and duration of steady-state inhibition of COX-1 activity and platelet function ex vivo by naproxen sodium at 220 and 440 mg BID or low dose aspirin for 6 days. An inhibition of platelet COX-1 activity ex vivo, as assessed by the measurement of serum TxB2, in six healthy subjects. The open symbols represent the values detected in each individual, whereas the closed symbols represent the mean ± S.D. §, P < 0.05 versus naproxen at 440 mg BID at corresponding times; *, P < 0.05 and, ** P < 0.01 versus aspirin at 24 h; broken line indicates inhibition of platelet COX-1 activity by 97%. Using a nonparametric test, #, P < 0.05 versus aspirin 24 h. B, serum TxB2 levels detected in each individual up to 24 h after dosing with the different treatments (open symbols). The colored closed symbols represent the mean ± S.D. The broken line indicates the serum TxB2 value of 10 ng/ml, *, P < 0.05 and, ** P < 0.01 versus aspirin at 24 h. C, inhibition of AA-induced platelet aggregation detected in six healthy subjects up to 24 h after dosing with the different treatments. The open symbols represent the degree of inhibition of platelet function detected in each individual, whereas the colored closed symbols represent the mean ±S.D. f, P < 0.05 versus aspirin at 1 h; *, P < 0.05 versus aspirin at 24 h.
Bayer sponsored study 12110 evaluated the platelet inhibitory effects of naproxen sodium at both OTC and prescription doses, in comparison to low dose enteric-coated aspirin. In this study, 48 subjects received either 550 mg naproxen sodium BID, 220 mg naproxen sodium BID, 220 mg naproxen sodium TID, or placebo for 7 days. After a minimum washout of 6 days, all subjects were then administered enteric-coated aspirin 81 mg QD for an additional 7 days. After 7 days of treatment with naproxen sodium, mean serum TxB2 inhibition (measured 24 h following the day 7 morning dose) was 97.9% for 220 mg BID, (lower bound of the 95% CI: 96.1) and 99.4% for 220 mg TID (lower bound of the 95% CI: 98.9), indicating significant thromboxane inhibition for both OTC dosing regimens (Figure 3). These data appear at odds with the Capone study which showed greater loss of inhibition post last dose.

Figure 3: Shiff Study – Day 7 Serum Thromboxane Inhibition measured 24 h following the day 7 morning dose

Results from the three studies described above, demonstrate naproxen’s inherent ability to produce high levels of TxB2 inhibition throughout a typical OTC dosing interval.

3.3 Potential for Pharmacodynamic Interaction Between Aspirin and NSAIDs

A pharmacodynamic interaction in the inhibition of the platelet has been suggested in patients concurrently administered aspirin and certain NSAIDs. Even though aspirin irreversibly inhibits platelet COX-1, the plasma half-life of aspirin is very short, approximately 15-20 minutes, whereas the half-life of several non-selective NSAIDs is much longer, e.g. ibuprofen 2-4 hours; naproxen 12-17 hours. As such, upon concurrent administration with a non-
selective NSAID, aspirin has a limited time to bind to COX-1 and this binding may be interfered with by the concurrent NSAID. Since it is unlikely there will be any aspirin available in the circulation once the NSAID agent has been released from the binding site, COX-1 will remain uninhibited. The specific half-life of an NSAID will influence the duration of its ability to interfere with aspirin binding to COX-1.

Non-selective NSAIDs (such as ibuprofen and naproxen) interfere with the antiplatelet effect of low dose aspirin by competing for binding sites in close proximity to the aspirin binding site on the platelet’s COX-1. This interaction is attributed to steric hindrance at the active site of COX-1 by ibuprofen and similar NSAIDs, which prevents aspirin from binding and irreversibly acetylating a serine residue on COX-1. A single daily dose of ibuprofen before aspirin can interfere with inhibition of serum TxB2 and inhibition of platelet aggregation by aspirin, as can multiple daily doses of ibuprofen. Similar effects have been described with naproxen. In fact, a single 500 mg dose of naproxen administered at the same time as 100 mg immediate release aspirin reportedly interfered with the antiplatelet effect of aspirin. However, the recent Kontakt study did not find a reduction in serum thromboxane inhibition when a single dose of naproxen sodium 220 mg or 440 mg was administered to subjects taking 81 mg IR aspirin.

3.4 Published Naproxen/Aspirin Pharmacodynamic Studies

A pharmacodynamic interaction between naproxen and low dose aspirin has been previously reported. The inherent antiplatelet activity and long half-life of naproxen, however, complicates the interpretation of pharmacodynamic interaction between aspirin and naproxen. Naproxen sodium in OTC doses has been reported to inhibit serum TxB2 to a similar degree as low dose aspirin. At higher systemic exposure to naproxen, concurrent administration of OTC naproxen (220 mg TID) or prescription naproxen (550 mg BID) with low dose aspirin was associated with platelet inhibition at levels comparable to those observed following low dose aspirin alone. Furthermore, 220 mg naproxen sodium BID with the first daily dose 2 hours after immediate release aspirin 100 mg/day has been associated with a level of TxB2 inhibition similar to the level of TxB2 inhibition observed with aspirin alone.

3.4.1 Capone et al. (2005)

In a small two-part study, Capone et al. evaluated the potential interaction between prescription doses of naproxen and low dose aspirin. In part one of the study, subjects (n=4) underwent a six day aspirin run-in (Days 1-6), followed by two treatment periods, each six days long. During the first treatment period (Days 7-12), subjects took immediate release aspirin 100 mg two hours prior to their morning dose of naproxen 500 mg, which was administered twice daily. Following a washout period, subjects were administered the same study medications, with the order of morning dosing switched – naproxen two hours before aspirin (Days 27-32). Upon completion of the six day aspirin run-in period, an almost complete inhibition of serum TxB2 production (99±0.2%) and platelet aggregation (95±0.6%) was observed (Figure 4). This effect lasted up to 26 hours after administration of the last run-in dose of aspirin, and was not impaired by naproxen two hours after aspirin or 2 hours before aspirin, for an additional 6 days.
Figure 4: Capone 2005 Study – Serum TxB2, Platelet Aggregation and Urinary 11-dehydro-TxB2 after Six Days on Treatment

Mean inhibition of platelet cyclooxygenase-1 activity ex vivo, as assessed by the measurement of serum thromboxane (Tx)B2 levels (A), arachidonic acid-induced platelet aggregation ex vivo (B), and Tx biosynthesis in vivo, as assessed by the measurement of urinary 11-dehydro-TxB2 levels (C), in subjects taking low dose aspirin alone (100 mg daily) for six days (hatched bars) and then re-administered with naproxen (500 mg twice daily, with the first dose administered 2 h after aspirin) for further six days (open bars). The solid bars show the effects of the same medications administered in reversed order for further six days after a washout period of 14 days. Values are reported as mean ± SEM, n = 4. All times are hours after the administration of the first study drug. Open bars = aspirin before naproxen (twice daily); solid bars = naproxen (twice daily) before aspirin; hatched bars = aspirin.

The second part of the study assessed serum COX-1 activity as measured by serum TxB2 and platelet aggregation for up to 14 days following a single co-administration of naproxen 500 mg and low dose immediate release aspirin. One hour after dosing, serum TxB2 and AA-induced platelet aggregation values were 92 ± 5% and 99 ± 0.1% of baseline levels, respectively. Serum TxB2 and platelet aggregation reached significant inhibition versus pre-drug values, beginning at the 3 hour time point and extending through the 48 hour time point (Figure 5).
Figure 5: Capone 2005 Study – Serum TxB2 and Platelet Aggregation over 14 Days after a single dose of aspirin 100 mg and naproxen 500 mg

Mean inhibition of platelet COX-1 activity ex vivo (solid circles) as assessed by measurement of serum TxB2 and AA-induced platelet aggregation ex vivo (open circles) in subjects taking a single dose for aspirin 100 mg and naproxen 500 mg. Values are reported as mean ± s.e.m., n=5.

Because aspirin’s peak antiplatelet effect is expected within approximately 1 hour of dosing, the delay in achievement of significant TxB2 suppression until the 3-hour time point, coupled with the rapid recovery of platelet COX-1 activity and function led the authors to conclude that naproxen interferes with the irreversible COX-1 inhibition afforded by aspirin. However, this effect was not evident during the continuous and regular concurrent administration of naproxen (500 mg BID) because naproxen can produce its own inhibitory effect on platelet TxA2 generation and possibly overshadow the interference with aspirin’s inhibitory effect on platelet TxA2 generation.

3.4.2 Anzelotti et al. (2011)

In another study, Anzelotti et al. investigated the potential for naproxen sodium, administered at OTC doses (220 mg twice a day) to interfere with the antiplatelet activity of low dose immediate release aspirin in 9 healthy volunteer subjects. This study consisted of three treatment periods, six days in length, separated by 14-day washout periods. An aspirin-only run-in was not used in this study. All assigned study medication was started on Day 1 of each treatment period. During the first treatment period, aspirin was administered two hours prior to naproxen sodium, which was then given again 12 hours later. During the second treatment period, the order of morning naproxen sodium and aspirin administration was reversed, and the final treatment period consisted of aspirin alone. On Day 6 of each treatment period, subjects were given only their morning doses of medication. Almost complete inhibition of COX-1 activity was observed 1 hour post-dose in the aspirin only group (99.8% [95% CI 99.7-99.9]). Naproxen before aspirin, aspirin before naproxen, and aspirin alone, all showed Day 6 24-hour post-dose serum TxB2 inhibition >95% (95.9% [95% CI 93.4-98.8%]; 98.9% [95% CI 98.4-99.4]; and 98.9% [95% CI 98.4-99.5%] respectively), though the level of inhibition observed for naproxen first was significantly lower than the other two regimens (Figure 6).
These data suggest that administration of aspirin 2 hours before naproxen minimizes the pharmacodynamic interaction between these two drugs, allowing for maintenance of thromboxane inhibition >95% lower bound of the 95% CI during the course of concurrent therapy.

![Figure 6: Anzellotti Study 2011](image)

3.4.3 Oldenhof et al. (2010)

Oldenhof et al.\(^7\) (also known as Bayer 12611) examined the effect on platelet function when 81 mg enteric-coated aspirin is taken concurrently with maximum OTC daily doses of naproxen sodium (220 mg TID) or acetaminophen (1000 mg QID). Thirty-seven subjects completed this three-period, parallel group trial, 12-13 per group. Days 1-5 of this study consisted of an aspirin-only run-in, which was followed by randomization into one of three treatment groups: continuation of aspirin alone, aspirin plus naproxen sodium TID, or aspirin plus acetaminophen QID. Each day’s initial dose of naproxen sodium or acetaminophen was taken at the same time as the daily dose of aspirin. Treatment was administered on Days 6-10, at which point subjects in the aspirin plus naproxen group entered an additional two day exploratory treatment period (Days 11-12) with aspirin alone.
All subjects first received 5 days of enteric-coated aspirin (EC-ASA) 81 mg once daily (QD) (Period 1, Days 1-5). On Days 6-10, were then randomized in a 1:1:1 ratio to receive 5 days of treatment with either EC-ASA 81 mg QD alone, EC-ASA 81 mg QD + acetaminophen 1000 mg four times daily (qid), or EC-ASA 81 mg QD + NAPSO 220 mg three times daily (tid). Blood sampling for determination of serum thromboxane B2 (TxB2) was drawn prior to the morning dose on Day 11.

TxB2 inhibition on Day 11 (12 hours following the last dose of naproxen sodium) was 99.7% (range 99.2–100%) in subjects given enteric coated-aspirin 81 mg and naproxen sodium 220 mg TID. For the Day 12, hour 12 exploratory endpoint, TxB2 inhibition was $\geq 95\%$ in 9 out of 11 subjects (98%; range 95.5-99.8%), though there were two subjects in whom inhibition dropped precipitously (0% and 33%) for unknown reasons.

### 3.5 Other Studies

Other investigators examined the effect of concurrent use of naproxen with low dose aspirin on shear-induced platelet function, using the closure time (CT) assessed with the platelet function analyzer (PFA-100). This assay exposes whole blood to shear through an aperture and measures the length of time before platelet aggregate formation is robust enough to close the aperture and stop blood flow. A prolonged closure time indicates a higher degree of platelet inhibition. Gailliard-Grigioni et al\(^39\) studied the co-administration of naproxen 250 mg with low dose enteric-coated aspirin. Administration of naproxen with low dose enteric coated aspirin significantly prolonged CT after 24 hours as compared to aspirin alone, indicating greater platelet inhibition ($P<0.001$). In contrast, Meek et al.\(^29\) observed small CT shifts with naproxen plus aspirin compared to aspirin plus placebo, suggesting a potential interference with the platelet inhibitory effect of aspirin.
4. **KONTAKT STUDY**

FDA advised Bayer that the results from published literature as well as study 12611 do not rule out the possibility of a drug interaction between aspirin and naproxen and requested that Bayer perform an additional pharmacodynamic drug interaction study with naproxen sodium and low dose aspirin. Specifically, FDA recommended an appropriately designed drug interaction study using immediate release aspirin and the lowest OTC dose and dosing regimen of naproxen sodium. FDA asserted that pharmacodynamic assessments should be conducted throughout the 24-hour dosing interval on the first day of concurrent naproxen sodium and low dose aspirin dosing and at steady state. Bayer selected 10 days of concurrent dosing for the steady state endpoint as it represents the maximum recommended dosing duration for OTC analgesics. FDA also stated that the reduction in serum TxB2 and platelet aggregation should be measured and compared. FDA provided further guidance on the study design, including treatment groups, treatment duration, pharmacodynamic assessments and time points.

While low dose aspirin is also available in an enteric coated formulation, the majority of studies investigating potential pharmacodynamic interaction between low dose aspirin and NSAIDs use immediate release aspirin, which has a highly predictable pharmacokinetic profile. Plasma half-life of immediate release aspirin is very short, approximately 15-20 minutes and repeat daily dosing produces a profound (>98%) thromboxane inhibition. Enteric coating delays absorption of aspirin and could increase the variability in the pharmacodynamic assessments being studied.

The aim of the Kontakt study, therefore, was to investigate whether concurrent administration of immediate release naproxen sodium 220 mg QD or BID and low dose immediate release aspirin (IR ASA) 81 mg QD results in a pharmacodynamic interaction. The interval between naproxen and aspirin dosing was also examined to determine whether timing influenced a potential pharmacodynamic interaction.

4.1 **Methods**

The investigation was a randomized, controlled, open-label, six-arm, parallel-group study (NCT02229461) that consisted of three sequential periods after screening: A) a 6-day, 81mg QD IR ASA run-in period (Days 1–6), B) a 10-day concurrent treatment period, where 220 mg QD or BID IR naproxen was added to 81mg QD IR ASA therapy (Days 7–16), and C) a 3-day 81mg QD IR ASA alone run-out period (Days 17–19) (Figure 8). Throughout the investigation, all subjects received 81 mg IR ASA each day.
4.1.1 Subjects

Healthy male and non-pregnant female volunteers providing written informed consent and aged 18–70 years were eligible. Exclusion criteria included: allergic reactions to aspirin, naproxen sodium, or other NSAIDs; a viral infection within one month prior to the start of the run-in period; a history of gastrointestinal complications from NSAID therapy, or a history of recurrent peptic ulcer/hemorrhage; medications including NSAIDs, vitamin or herbal supplements within 7 days, or antiplatelet or anticoagulant drugs within 30 days prior to the start of the run-in period or throughout the study; donation of blood or blood components within 30 days prior to study entry; recent surgery, or planned surgery or invasive procedure, and smokers or anyone who consumed any type of nicotine or tobacco products.

Within 28 days of the screening visit for eligibility, subjects returned to the clinical study site for Day 1 of the run-in period where they were administered their first, single dose of 81 mg IR ASA (Figure 8). The doses on Days 2 and 3 were taken at home and subjects were instructed to take aspirin in the morning (08:00) with a full glass of water after a fast of at least 10 hours, and to refrain from meals, snacks and fluids for 1 hour after dosing. The remaining three doses (Days 4–6) were administered at the clinical study site to ensure compliance.
Subjects who demonstrated a Day 1 (baseline, pre-aspirin) serum TxB2 ≥5000pg/mL, and who took aspirin for at least five out of six days (including the Day 6 dose), and who had 2mM AA-induced maximal platelet aggregation <20% on Day 7 were considered eligible to continue in the study. These subjects were then randomized and sequestered at the clinical study site for 14 days, and the study drugs were administered by the clinical study site staff. Treatment was administered for 10 consecutive days (Days 7-16), followed by a 3-day 81mg QD ASA-only run-out period for all groups (Days 17-19). Subjects were discharged on Day 20.

4.1.2 Treatments

The treatments administered were IR ASA 81mg chewable tablets (Bayer®) QD and IR naproxen sodium 220 mg caplets (Aleve®) QD or BID. Eligible subjects were randomized with equal chance to one of six treatment groups (Figure 8) according to a pre-generated randomization list. Personnel performing the analyses were blinded to treatment assignment.

4.1.3 Pharmacodynamic assessments

Serum TxB2, platelet aggregation and PRP TxB2 were assessed at baseline, first day of concurrent dosing (Study Day 7), tenth day of concurrent dosing (Study Day 16), first day of aspirin only run out (Study Day 17) and third day of aspirin only run out (Study Day 19) of the in-house treatment period at 1, 3, 6, 12, 18, and 24 hours post-dose relative to the time of IR ASA 81mg administration.

Inhibition of serum TxB2 measured 24 hours after aspirin administration on the tenth day of concurrent dosing (Study Day 16) was the primary endpoint. Secondary endpoints included inhibition of serum TxB2 at multiple time points on Study Days 7, 16, 17 and 19. An exploratory pharmacodynamic assessment was inhibition of TxB2 in platelet-rich plasma (PRP) following stimulation with AA, also on Days 7, 16, 17, and 19 of the treatment period.

Blood samples for TxB2 levels were collected by venipuncture. For serum TxB2 measurements, samples were collected in glass serum tubes (BD Vacutainer Tubes). After gentle inversion, the tubes were immediately incubated in a 37 °C water bath for 60-65 minutes then centrifuged at 4 °C for 10 minutes at 2000 g to separate serum. The resultant serum was immediately transferred to cryovials to be stored at -70°C before shipping for analysis. Blood samples for platelet aggregation and PRP TxB2 were collected in citrate tubes and centrifuged at 250 g for 10 minutes to recover platelet-rich plasma (PRP). PRP was stimulated by adding 2 mM AA and maximal platelet aggregation was assessed with a Chrono-log model 570VS (Chrono-log Corp, Havertown, PA, USA) aggregometer. Following aggregation, platelet rich plasma was centrifuged at 4 °C for 8 minutes at 4000 g and the supernatant PRP was immediately transferred to a cryovial to be stored at -70 °C before shipping for analysis.

Serum samples were measured for TxB2 by an enzyme linked immunoassay method (Thromboxane B2 ELISA Kit, Cayman Chemical Company, Ann Arbor, MI, USA; Catalogue no. 501020) according to manufacturer’s instructions. TxB2 levels were assayed by Northeast Bioanalytical Laboratory. Similarly, supernatant PRP TxB2 was assessed using a similar method (Thromboxane B2 ELISA Kit, Cayman Chemical Company, Ann Arbor, MI, USA; Catalogue no. 501020).
4.1.4 Statistical Methods

Baseline was defined as the last non-missing assessment (including repeated and unscheduled assessments) before the first dose of low dose aspirin on Day 1. Subjects who did not demonstrate at least 98% serum TxB2 inhibition at pre-dose on Day 7 (first day of concurrent treatment) or who did not provide serum TxB2 inhibition data at 24 hours on Day 16 were excluded from the statistical analyses for the evaluable population. The evaluable population was used to perform the assessment of the potential pharmacodynamic interaction between naproxen and aspirin. All statistical analyses were conducted using SAS Version 9.2.

Inhibition of serum TxB2 and PRP TxB2 at each time point was calculated using the percent reduction from baseline (at pre-dose on Visit 2, Day 1) as follows: inhibition (%) = 100 × (baseline value – post-dose value) / baseline value.

For the primary pharmacodynamic analysis, the mean and lower bound of the corresponding one-sided 95% confidence interval (CI) for the serum TxB2 inhibition at 24 hours on Study Day 16 (last day of concurrent treatment) for each treatment group were calculated.

A pharmacodynamic interaction was defined to occur when the lower bound of the one-sided 95% CI for serum TxB2 inhibition was below 95% at 24 hours on Study Day 16 (last day of concurrent treatment). Serum TxB2 inhibition at all other time points was analyzed similarly as the secondary analysis. PRP TxB2 inhibition was analyzed on an exploratory basis. The threshold of 95% inhibition was used to define a pharmacodynamic interaction and was based on the demonstration that this high level of ex vivo inhibition is required for in vivo inhibition as determined by measurement of urinary 11-dh TxB2.

4.2 Results

4.2.1 Subject Disposition

One hundred and seventeen subjects were enrolled in the run-in period and 102 subjects were randomized on Day 7 and enrolled into the treatment period (Figure 9). Twenty-two subjects with < 98% serum TxB2 inhibition at pre-dose on Day 7 were excluded resulting in 80 subjects included in the evaluable population. The average (±std. dev) % serum TxB2 among these 22 subjects was 95.6 ± 4.7% (range 78.52% – 97.97%).
IR ASA = immediate release aspirin, AA = arachidonic acid. TxB2 = thromboxane B2
Group 1: IR ASA 81 mg once daily (QD) plus naproxen sodium 220 mg QD administered at the same time;
Group 2: IR ASA 81 mg QD administered 30 minutes after naproxen sodium 220 mg QD;
Group 3: IR ASA 81 mg QD administered 8 hours after naproxen sodium 220 mg QD;
Group 4: IR ASA 81 mg QD only (reference group);
Group 5: IR ASA 81 mg QD administered 30 minutes before naproxen sodium 220 mg QD;
Group 6: IR ASA 81 mg QD administered 30 minutes after the first dose of naproxen sodium 220 mg BID (dosing 12 hours apart)

4.2.2 Pharmacodynamic Analysis
4.2.2.1 Primary Analysis
In all of the concurrent treatment groups there was an interaction at 24 hours post-dose on Day 16 (Figure 10; Table 1).
Group 1: IR ASA 81 mg once daily (QD) plus naproxen sodium 220 mg QD administered at the same time;
Group 2: IR ASA 81 mg QD administered 30 minutes after naproxen sodium 220 mg QD;
Group 3: IR ASA 81 mg QD administered 8 hours after naproxen sodium 220 mg QD;
Group 4: IR ASA 81 mg QD only (reference group);
Group 5: IR ASA 81 mg QD administered 30 minutes before naproxen sodium 220 mg QD;
Group 6: IR ASA 81 mg QD administered 30 minutes after the first dose of naproxen sodium 220 mg BID (dosing 12 hours apart)
Table 1: Summary of serum TxB₂ inhibition at 24 hours post-dose on Day 16 (last day of concurrent treatment) (evaluable population)

<table>
<thead>
<tr>
<th>Serum TxB₂ inhibition (24 hours post-dose on Day 16)</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>N</td>
<td>13</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>93.1±3.20</td>
</tr>
<tr>
<td>Min, max</td>
<td>85.7, 97.9</td>
</tr>
<tr>
<td>Lower bound of 1-sided 95% CI of mean</td>
<td>91.50</td>
</tr>
<tr>
<td>Two-sided 95% CI of mean</td>
<td>(91.15, 95.02)</td>
</tr>
<tr>
<td>n (%) subjects with inhibition ≥95%</td>
<td>3 (23.1)</td>
</tr>
<tr>
<td>n (%) subjects with inhibition ≥90%</td>
<td>11 (84.6)</td>
</tr>
</tbody>
</table>

CI, confidence interval; SD, standard deviation.
Group 1: IR ASA 81 mg once daily (QD) plus naproxen sodium 220 mg QD administered at the same time;
Group 2: IR ASA 81 mg QD administered 30 minutes after naproxen sodium 220 mg QD;
Group 3: IR ASA 81 mg QD administered 8 hours after naproxen sodium 220 mg QD;
Group 4: IR ASA 81 mg QD only (reference group);
Group 5: IR ASA 81 mg QD administered 30 minutes before naproxen sodium 220 mg QD;
Group 6: IR ASA 81 mg QD administered 30 minutes after the first dose of naproxen sodium 220 mg BID (dosing 12 hours apart)
4.2.2.2 Secondary Analyses

4.2.2.2.1 Serum TxB2 inhibition

When aspirin was administered alone (Group 4, aspirin only group), the lower bound of the mean serum TxB2 inhibition was >98% on all days and all time points (Figure 11). Throughout the 24 hours following aspirin administration on the first day of concurrent dosing (Day 7), TxB2 levels remained above 95% inhibition at all time points in all groups.

After 10 days of concurrent dosing (Day 16), the lower bound of the mean serum TxB2 inhibition remained ≥95% for all concurrent treatment groups for at least 6 hours after dosing with aspirin but declined over the 24-h period. The lowest percent inhibition of serum TxB2 was seen at the 24-h time point. The lower bound remained ≥94% at all Day 16 time points in subjects who received aspirin 30 minutes before naproxen QD (Group 5) or aspirin 30 minutes after the first dose of naproxen BID (Group 6) (Figure 11). Subjects in Group 2, who received aspirin 30 minutes after naproxen QD, showed the greatest decline in serum TxB2 inhibition at 24 hours, as reflected in the 2-sided 95% CI that did not overlap with any other group (Table 1).

In the first day of the aspirin only run-out period (Day 17), the lower bound of the mean serum TxB2 inhibition fell below 95% at multiple time points in all concurrent treatment regimens (Figure 11). The lowest inhibition was seen at the 24 hour time point in Group 2 (aspirin administered 30 minutes after naproxen QD) (81.77%) and Group 6 (aspirin administered 30 minutes after the first dose of naproxen BID) (80.66%). By the third day of the aspirin only run out (Day 19), the lower bound of serum TxB2 inhibition was >95% at all time points in Groups 1 (aspirin and naproxen administered concurrently), 2 (aspirin 30 minutes after naproxen QD), and 3 (aspirin administered 8 hours after naproxen QD) and >95% in all time points except 24 hours (94.86%) in Group 5 (Figure 11). In Group 6 (aspirin 30 minutes after the first dose of naproxen BID), a persistent interaction was observed on Day 19 over the 24-h period.

4.2.2.2.2 TxB2 Inhibition in PRP

PRP TxB2 inhibition showed a similar trend as serum TxB2 inhibition with generally lower inhibition across all time points in each treatment group in the evaluable populations.
Figure 11: Inhibition of serum TxB2 (%) at each time point in each treatment group. Mean and one-sided 95% confidence interval by treatment group (evaluable population)
4.2.2.3 Platelet Aggregation

A broad range of baseline AA-induced platelet aggregation, including some low baseline values, contributed to considerable variability in the post-baseline inhibition results. To compensate, median values and other non-parametric descriptive statistics were examined. In general, less inhibition of AA-induced platelet aggregation was observed at all time points when naproxen sodium 220 mg was administered with IR ASA 81 mg, compared with IR ASA 81 mg administered alone. At the end of the IR ASA Run-In (Day 7 pre-dose), median values for the percent inhibition of AA induced platelet aggregation ranged from 87.5% to 90.79% across the 6 treatment groups. On the first day of concurrent dosing (Study Day 7), the median percent inhibition of AA-induced platelet aggregation remained above 80% in all treatment groups over all Day 7 assessment times; however, only Group 4 (IR ASA alone) remained above 90% at all Day 7 time points. At the end of the 10 day concurrent treatment period (Study Day 16), the Hour 24 median percent inhibition of AA induced platelet aggregation was 89.26, 89.20, 96.60, 93.84, 85.60 and 87.51, in Groups 1 through 6, respectively (Table 2). Small differences between groups were seen in median percent inhibition of AA-induced platelet aggregation on Day 16 but remained in all groups above 85% at all time points; however, only Group 4 (IR ASA alone) produced a median value above 90% at all Day 16 observations. Similarly during the 3-day aspirin Run Out, small differences between groups were seen in median percent inhibition of AA induced platelet aggregation but remained in all groups above 83% at all time points. Again, only Group 4 (IR ASA alone) consistently produced a median value above 90% (except Day 17 Hour 1; 89.47%).

Table 2: Summary of Inhibition of Arachidonic Acid-Induced Platelet Aggregation (median) Evaluable Population

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (N=13)</th>
<th>Group 2 (N=14)</th>
<th>Group 3 (N=15)</th>
<th>Group 4 (N=13)</th>
<th>Group 5 (N=10)</th>
<th>Group 6 (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DAY 7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predose</td>
<td>87.50</td>
<td>89.29</td>
<td>90.00</td>
<td>90.79</td>
<td>90.63</td>
<td>88.66</td>
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<tr>
<td><strong>DAY 7</strong></td>
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<td></td>
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<tr>
<td>24 HR</td>
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<tr>
<td>24 HR</td>
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<td>96.60</td>
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<tr>
<td><strong>DAY 17</strong></td>
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</tr>
<tr>
<td>24 HR</td>
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<td>24 HR</td>
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<td>93.50</td>
<td>94.23</td>
<td>92.34</td>
<td>96.74</td>
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</tbody>
</table>

Group 1: IR ASA 81 mg once daily (QD) plus naproxen sodium 220 mg QD administered at the same time;
Group 2: IR ASA 81 mg QD administered 30 minutes after naproxen sodium 220 mg QD;
Group 3: IR ASA 81 mg QD administered 8 hours after naproxen sodium 220 mg QD;
Group 4: IR ASA 81 mg QD only (reference group);
Group 5: IR ASA 81 mg QD administered 30 minutes before naproxen sodium 220 mg QD;
Group 6: IR ASA 81 mg QD administered 30 minutes after the first dose of naproxen sodium 220 mg BID (dosing 12 hours apart)
4.2.2.4 Thromboxane in Platelet Rich Plasma

Aside from platelets, macrophages and monocytes are other potential sources of TxB2 in serum. Thromboxane B2 in PRP, however, is a more direct reflection of platelet derived TxB2. The high degree of thromboxane inhibition found in the plasma thromboxane is consistent with the inhibition of serum TxB2 and is indicative of the pharmacologic inhibition of platelet function by aspirin. The summary of plasma TxB2 inhibition showed a similar trend as observed in the summary of serum TxB2 inhibition with generally lower values across all time points in each treatment group. At the end of the IR ASA Run-In Period, mean values for the percent inhibition of plasma TxB2 ranged from 94.32% to 95.85% across the 6 treatment groups and the lower bound of the 95% CI ranged from 92.80% to 94.64% across the treatment groups. On the first day of concurrent dosing (Study Day 7), the mean percent inhibition of plasma TxB2 remained above 95% in all treatment groups over all Day 7 assessment times, and the lower bound of the 95% one-side CI remained above 93%, with Group 4 (IR ASA alone) producing the lowest value, 93.34%, at Hour 24. At the end of the 10 day concurrent treatment period (Study Day 16), the Hour 24 mean percent inhibition of plasma TxB2 was 86.42, 72.66, 77.71, 95.78, 88.49, and 86.87, in Groups 1 through 6, respectively (Table 3). Only Group 4 achieved a mean percent inhibition above 95% and lower confidence bound above 90% at all of the follow-up time points. Subjects in groups receiving IR ASA prior to naproxen sodium or naproxen sodium BID showed the least drop-off in plasma thromboxane inhibition over 24 hours relative to Group 4, while subjects in Group 2 showed the most. During the IR ASA Run-Out Period, Groups 3 and 5 showed the least decline relative to Group 4. The largest decline on Day 19 was seen in Group 6.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (N=13)</th>
<th>Group 2 (N=14)</th>
<th>Group 3 (N=15)</th>
<th>Group 4 (N=13)</th>
<th>Group 5 (N=10)</th>
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<tr>
<td>DAY 7</td>
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<tr>
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<td>95.81</td>
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<tr>
<td>DAY 7 24 HR</td>
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<td>96.63</td>
<td>96.14</td>
<td>81.67</td>
</tr>
</tbody>
</table>

Group 1: IR ASA 81 mg once daily (QD) plus naproxen sodium 220 mg QD administered at the same time;
Group 2: IR ASA 81 mg QD administered 30 minutes after naproxen sodium 220 mg QD;
Group 3: IR ASA 81 mg QD administered 8 hours after naproxen sodium 220 mg QD;
Group 4: IR ASA 81 mg QD only (reference group);
Group 5: IR ASA 81 mg QD administered 30 minutes before naproxen sodium 220 mg QD;
Group 6: IR ASA 81 mg QD administered 30 minutes after the first dose of naproxen sodium 220 mg BID (dosing 12 hours apart)
5. DISCUSSION

The Kontakt study was rigorously designed to maximize the potential for observing a pharmacodynamic interaction of concurrent treatment of naproxen sodium and aspirin. The pharmacodynamic threshold for declaring no interaction was defined as $\geq 95\%$ for the lower bound of the one-sided 95% CI for serum TBx2 inhibition.

The study demonstrated that:

- Throughout the 24 hours following aspirin administration on the first day of concurrent dosing, greater than 95% TxB2 inhibition was maintained in all treatment groups;
- After 10 days of concurrent dosing, greater than 95% TxB2 inhibition was maintained for at least 6 hours following 81mg IR ASA dosing, but fell below the 95% threshold thereafter; and
- During the run-out period when aspirin was administered alone following concurrent treatment for 10 days, TxB2 inhibition fell below the 95% threshold at multiple time points in all of the concurrent treatment groups. Similar to the concurrent treatment phase, the effect was less evident in patients administered aspirin 30 minutes before naproxen and it appeared more pronounced in patients administered aspirin 30 minutes after naproxen. After completion of a 10-day course of concurrent use, the pharmacodynamic interaction persisted after discontinuing naproxen for fewer than 3 days among subjects administered aspirin plus once daily naproxen and for at least 3 days among subjects treated with low dose aspirin plus twice daily naproxen sodium 220 mg.

The influence of the sequence and timing of administration of naproxen and IR ASA on the pharmacodynamic interaction observed in the Kontakt study may be explained by the inhibition of COX-1 by naproxen and a 12-17 hour half-life. A high degree of serum TxB2 inhibition was maintained during concurrent dosing for at least 6 hours in all groups on Day 16, and remained above 90% at all time points, except at 24 hours in Group 2 (85.51%). Furthermore, no interaction was observed for at least 18 hours in subjects administered aspirin 30 minutes before naproxen. The number of days of concurrent dosing before less than 95% TxB2 inhibition is observed cannot be determined from this study as no samples were collected on Days 2-9 of concurrent dosing.

A pharmacodynamic interaction was not observed on the first day of concurrent treatment (Day 7). These findings suggest that in individuals on a low dose aspirin regimen, use of a single dose of naproxen sodium would not impact TxB2 inhibition. However, TxB2 inhibition was not studied on the days between the first (Day 7) and tenth (Day 16) day of concurrent dosing so the number of consecutive days of dosing with these concurrent treatment regimens before TxB2 inhibition falls below the 95% threshold cannot be determined from this study.

Naproxen has a long half-life compared to the very short half-life of aspirin (15-20 min). Therefore, upon concurrent administration, aspirin may not be remaining in the circulation to inhibit COX-1 once naproxen has been released from the platelet binding site during the latter part of the day. Conversely, daily aspirin may be introduced to the circulation at a time when platelet COX-1 is already reversibly inhibited by naproxen, thus preventing aspirin from binding. An interaction with the anti-platelet effect of aspirin is likely to persist until naproxen has been eliminated from the circulation and removed from the platelet binding site. Indeed, as observed in the Kontakt study on the first day of the aspirin run-out period (Day 17), an interaction was
observed at multiple time points in all of the concurrent treatment regimens. In the once-daily naproxen groups, by the third day of the aspirin run-out period (Day 19), the lower bound had increased to >95% inhibition in Groups 1, 2, 3) and 94.86% in Group 5 at 24 hours. These data demonstrate that any pharmacodynamic interaction with aspirin caused by once-daily naproxen resolves within three days. In the twice-daily naproxen group (Group 6), the pharmacodynamic interaction between naproxen and aspirin persisted on Day 19 but was less pronounced as compared to Day 17 (Figure 11). This finding suggests that higher levels of naproxen in the circulation due to twice daily dosing resulting in greater naproxen occupancy of COX-1 may be responsible for the prolonged interaction with aspirin.

The greatest decline in serum TxB2 inhibition occurred when aspirin was given after naproxen. These results support the findings from another study, where administration of aspirin 2 hours after naproxen led to a pharmacodynamic interaction and lower inhibition of serum TxB2 at 24 hours on Day 6 of treatment, whereas almost complete inhibition of TxB2 was noted when aspirin was taken 2 hours before naproxen. Thus it seems that the pharmacodynamic interaction may be diminished if immediate release aspirin is taken at least 30 minutes before naproxen sodium.

The proportion of subjects who maintained serum TxB2 inhibition ≥95% at all Day 16 time points was greater in subjects who received aspirin 30 minutes before naproxen QD (Group 5) or aspirin 30 minutes after the first dose of naproxen BID (Group 6) compared to the other groups that received concurrent therapy. Inspection of individual subject data, revealed that 50% of subjects (5 of 10) assigned to Group 5 and 53% of subjects (8 of 15) assigned to Group 6 maintained serum TxB2 inhibition ≥95% at all Day 16 time points compared to 15.38% (2 of 13), 0% (0 of 14) or 13.33% (2 of 15) of subjects in Groups 1, 2 and 3, respectively.

The concentration of TxB2 in platelet-rich plasma is a direct reflection of platelet-derived TxB2. The high degree of inhibition of TxB2 in PRP, an exploratory endpoint of our study, confirmed potent inhibition of platelet COX-1 activity.

6. CLINICAL IMPLICATIONS

A pharmacodynamic interaction in the inhibition of platelet activity has been observed in patients concurrently taking aspirin and certain NSAIDs. This pharmacodynamic interaction between aspirin and NSAIDs has raised concern regarding the cardioprotective efficacy resulting from high level COX-1 inhibition in patients with high-risk cardiovascular disease using low dose aspirin for cardioprotection. However, no clinical outcome studies are available upon which to assess the clinical implications of this pharmacodynamic interaction. One challenge with designing such studies is the lack of an established surrogate laboratory marker for cardioprotection. Although ≥95% inhibition of TxB2 is considered necessary for cardioprotection by some investigators, there is currently no standard that defines the optimal percent inhibition of serum TxB2 for prevention of secondary cardiovascular events. Moreover, there is variability in the literature on the appropriate threshold for serum TxB2 inhibition that is associated with adequate platelet inhibition. Some published studies recognized ≥90% TxB2 inhibition as the test threshold for adequate platelet inhibition and to assess an optimal antiplatelet effect of aspirin. The lower bound of 90% inhibition of TxB2 inhibition has
also been used to determine non-inferiority in drug interaction studies with aspirin and ibuprofen\textsuperscript{47}, as well as a recent study with the combination of esomeprazole and naproxen.\textsuperscript{48} However, other investigators have suggested that 95\% inhibition of baseline TxB2 is necessary for adequate cardioprotection, and 95\% TxB2 inhibition ex vivo correlates with the inhibition of in vivo TxA2 generation (Reilly, 2017). The variability in these suggested thresholds would seem to imply that a slight decline in TxB2 inhibition is unlikely to have clinical significance. The FDA has previously acknowledged $\geq 90\%$ TxB2 inhibition as the criterion used by several investigators to assess the clinical significance of drug interaction with aspirin\textsuperscript{5} but subsequently requested a 95\% threshold in the Kontakt study.

The clinical relevance of this pharmacodynamic interaction remains unknown. No large, randomized, well-controlled clinical outcome trials designed specifically to address this endpoint have been conducted. Reviews of CV safety studies involving prescription strength NSAIDs and aspirin have been mixed. As noted in a review by Nalamachu et al.\textsuperscript{49}, the evidence in the clinical literature is conflicting. One large retrospective study found no evidence of reduced cardioprotective effects of aspirin when taken with NSAIDs.\textsuperscript{50} However, in a large study of patients taking low dose aspirin, those who also took ibuprofen had a higher rate of recurrent acute myocardial infarction and those who took aspirin and naproxen had a lower rate of recurrent acute myocardial infarction compared with patients who took only low dose aspirin.\textsuperscript{51} Farkouh et al.\textsuperscript{52} reported that patients with cardiovascular disease taking aspirin and some NSAIDs appear to have increased vascular events. Other reviews\textsuperscript{53,54} did not find an increased CV risk among patients concurrently using long term aspirin and NSAIDs. Similarly, observational studies by Hawkey et al.\textsuperscript{55} and Rahme et al.\textsuperscript{56}, which including a naproxen-aspirin cohort or subgroup, reported no significant difference in rates of myocardial infarction in patients using naproxen with low-dose aspirin. A post hoc analysis of the recent PRECISION study examined the effect of concurrent aspirin and NSAID use on major adverse CV events (MACE) and found no difference between naproxen and celecoxib among aspirin users.\textsuperscript{57} As celecoxib does not exert a pharmacodynamic interference with aspirin, this observation may suggest that any potential pharmacodynamic interaction seen with naproxen does not adversely affect the cardioprotection afforded from daily aspirin use. The PRECISION trial and this subanalysis studied neither the lower OTC naproxen sodium dose, nor the labeled direction of occasional, short-term use.

Finally, in more than 20 years of safety data monitoring and availability as a non-prescription pain reliever, pharmacovigilance data indicate no signal or trend with regard to the occurrence of CV thrombotic and overall CV events.

7. CONCLUSION

Aspirin’s antiplatelet property is attributed primarily to irreversible acetylation of the platelet COX-1 enzyme, and subsequent inhibition of thromboxane generation. In contrast to aspirin, which inhibits platelet COX-1 for the lifetime of the platelet, non-selective naproxen sodium, and other non-selective NSAIDs bind reversibly to COX-1 and provide a temporary antiplatelet effect. Despite this difference, multiple-day dosing with OTC 220 mg immediate-release naproxen administered two or three times per day reduced serum TxB2 to a similar degree as low-dose (81 mg/day) aspirin over a 24-hour period.

Current evidence indicates a pharmacologic interaction between naproxen and low dose aspirin when the two drugs are administered concurrently. However, the interaction is variable and
depends on several factors, including, dose, duration and timing of concurrent naproxen and aspirin use. There is some evidence that interference may be minimized by specific dosing schedules of NSAIDs and aspirin. For example, a singular dose of naproxen may have no pharmacodynamic effect. With multiple day exposure, administering naproxen sodium 220 mg BID with the first daily dose 2 hours after IR aspirin can maintain >95% TxB2 inhibition for 24 hours after the last dose. At higher systemic exposure to naproxen, concurrent administration of OTC naproxen (220 mg TID) or prescription naproxen (550 mg BID) with low dose aspirin shows platelet inhibition at comparable levels seen with low dose aspirin alone.

The Kontakt study investigated whether concurrent administration of low daily OTC doses of naproxen sodium, 220 mg once or twice daily, results in a pharmacodynamic interaction when combined with a low-dose (81 mg/day) immediate release aspirin regimen under specific dosing interval patterns and assessed whether timing influenced a potential pharmacodynamic interaction. The study was rigorously designed to maximize the potential for observing an interaction and the pharmacodynamic threshold for declaring no interaction was defined as ≥95% for the lower bound of the one-sided 95% CI for serum TxB2 inhibition.

Results of the Kontakt study show:

- Evidence of a pharmacodynamics interaction was not observed during the first day of concurrent treatment. These findings suggest that in individuals on a low dose aspirin regimen, use of a single dose of naproxen sodium may not impact TxB2 inhibition.

- After 10 days, irrespective of the timing and dose of naproxen in relation to aspirin dosing, a pharmacodynamic interaction was observed in all of the concurrent treatment groups. The number of days of concurrent dosing before TxB2 inhibition falls below the 95% threshold cannot be determined from this study as no samples were collected on Days 2-9 of concurrent dosing.

- The degree of pharmacodynamic interaction appears to be influenced by the timing of aspirin and naproxen dosing when used concurrently over multiple days. Subjects assigned to low dose aspirin 30 minutes before once daily naproxen sodium 220 mg achieved >94% thromboxane inhibition, narrowly falling short of the 95% threshold.

- After 10 days of concurrent use of low dose aspirin and naproxen sodium, evidence of a pharmacodynamic interaction persisted after discontinuing naproxen for fewer than 3 days among subjects administered 81 mg immediate release aspirin plus once daily naproxen sodium 220 mg and for at least 3 days among subjects treated with low dose aspirin plus twice daily naproxen sodium 220 mg.

- In the control group (aspirin alone), the lower bound for serum TxB2 inhibition was >98% at all time points.

The clinical relevance of this pharmacodynamic interaction remains unknown. Reviews of CV safety studies involving prescription strength NSAIDs and aspirin have been mixed. Published data on concurrent intake of prescription doses of naproxen and aspirin generally do not show an increase in CV events. However, no large, randomized, well-controlled clinical outcome trials designed specifically to address this endpoint have been conducted.

Naproxen sodium is a commonly-used and widely available OTC NSAID at doses up to 660 mg per day for up to 10 days. When taken as directed, it is a safe and effective OTC pain reliever to
temporarily relieve minor aches and pains, including that of arthritis, headache, backache, muscular aches, toothache and menstrual cramps, since its introduction as a self-care medication in 1994. In more than 20 years of safety data monitoring and availability as a non-prescription pain reliever, pharmacovigilance data indicate no signal or trend with regard to the occurrence of CV thrombotic and overall CV events.
8. REFERENCES


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