Dissertation

The Influence of Tramadol on Platelet´s Function

submitted by

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Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the „Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz“.

In the writing process of this thesis generative Artificial Intelligence (AI) (ChatGPT-4) was used as a language editing service to improve the readability and language of parts of the manuscript. No confidential information or results were disclosed to or shared with ChatGPT. All the output was carefully reviewed by myself, and I take full responsibility for the content of this thesis.

Graz , January 21st, 2026 Dr.med.univ. Philipp Zoidl eh

Disclosure

This thesis was written at the doctoral school “Neuroscience”.

Parts of this thesis have been published in the following article:

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Furthermore, parts of this dissertation were presented in a poster presentation at the Doctoral Day of the Medical University of Graz, in Graz, Austria (2024).

I declare that all co-authors consented to the inclusion of their data in this dissertation. Written statements are submitted together with the thesis. The reuse of this article in the present dissertation is permitted under the author rights granted by the publisher. The original publication in *Haemostaseologie* is fully acknowledged, and the material is reproduced in accordance with Thieme’s author reuse policy. No additional permission was required.

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Table of content

Abbreviations

ARA	Arachidonic acid
ASA	Acetyl-salicyl Acid
COX	Cyclooxygenase
EDTA	Ethylenediaminetetraacetic acid
GIT	Gastrointestinal tract
LKH	„Landeskrankenhaus“ = State Hospital
LTA	Light transmission aggregometry
MCF	Maximum clot formation
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
PRP	Platelet rich plasma
ROTEM	Rotational thromboelastometry
SAH	Subarachnoid hemorrhage
SERT	Serotonin transporter
SNRI	Serotonin–norepinephrine reuptake inhibitors
SSRI	Selective serotonin reuptake inhibitor
TRAP	Thrombin receptor activating peptide
TRPA1	Transient Receptor Potential Ankyrin 1
TXAS	Thromboxane synthase
WHO	World Health Organisation

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Abstract

Background and Aim

Tramadol is one of the most used analgesics worldwide and occupies an important role in perioperative and chronic pain management. Unlike many other opioids, tramadol has a distinctive pharmacodynamic profile, acting not only as a weak μ-opioid receptor agonist but also as a serotonin and norepinephrine reuptake inhibitor. These serotonergic properties raise questions about its potential influence on platelet function and coagulation, since serotonin is a key mediator of platelet aggregation and vascular tone. While the effects of NSAIDs, paracetamol, and metamizole on platelet activity are well documented, the impact of tramadol remains poorly understood and inconsistently reported. Previous studies have shown contradictory findings, ranging from enhanced aggregation to delayed clot formation, underscoring the need for systematic evaluation. The aim of this study was to investigate the effect of tramadol on platelet function at various concentrations, both alone and in combination with other drugs commonly administered concomitantly, such as metamizole, ibuprofen, and fentanyl.

Methods

This single-center laboratory study was conducted at the Medical University of Graz following ethics approval (IRB00002556) and ClinicalTrials.gov registration (NCT05237492). Thirty-three healthy adult volunteers were enrolled after informed consent. Exclusion criteria included anticoagulant therapy, history of coagulopathy, or ongoing therapy with opiates. Blood samples were collected and platelet-rich plasma prepared for analysis by light transmission aggregometry (LTA), the reference method for platelet function testing. Tramadol was titrated in concentrations ranging from 500 to 9000 ng/ml. Additional experiments tested tramadol (200 ng/ml) in combination with metamizole (300–900 μg/ml), ibuprofen (60–180 μg/ml), or fentanyl (3000–9000 ng/ml). Aggregation was induced by standard agonists including ADP, collagen, arachidonic acid, ristocetin, and TRAP. Statistical analyses included Friedman's test for non-parametric repeated measures, Kendall's W for effect size estimation, and post-hoc comparisons where appropriate.

Results

Baseline platelet aggregation values were consistent across controls. Tramadol alone, even at supratherapeutic concentrations, did not produce statistically significant changes in platelet aggregation with any agonist tested (ADP: p=0.284; collagen: p=0.382; TRAP: p=0.502; ristocetin: p=0.404; arachidonic acid: p=0.121). Effect sizes were small to moderate.

In contrast, tramadol combined with metamizole demonstrated significant reductions in platelet aggregation after ADP (p=0.015), collagen (p=0.016), and ristocetin activation (p=0.027), with large effect sizes (Kendall's W up to 0.775). Similarly, tramadol with ibuprofen produced significant inhibitory effects under ADP (p=0.021), arachidonic acid (p=0.013), and collagen activation (p=0.038). No statistically significant differences were observed with tramadol–fentanyl combinations, though trends toward reduced aggregation were noted in some assays (p-values 0.058–0.126).

Conclusion

In this ex vivo study, tramadol alone did not significantly alter platelet aggregation across multiple pathways, supporting the null hypothesis. However, when combined with metamizole or ibuprofen, tramadol was associated with an inhibitory effect on platelet function, whereas fentanyl showed no relevant interaction.

This potential interaction warrants consideration in perioperative pain management, especially in patients at elevated bleeding risk. Future clinical studies with larger sample sizes are necessary to confirm these results and to assess their translation into in vivo settings.

Zusammenfassung

Hintergrund und Zielsetzung

Tramadol gehört zu den weltweit am häufigsten eingesetzten Analgetika und spielt eine wichtige Rolle in der perioperativen sowie chronischen Schmerztherapie. Im Gegensatz zu vielen anderen Opioiden weist Tramadol ein besonderes pharmakodynamisches Profil auf: Es wirkt nicht nur als schwacher μ-Opioidrezeptor-Agonist, sondern auch als Serotonin- und Noradrenalin-Wiederaufnahmehemmer. Diese serotonergen Eigenschaften werfen Fragen hinsichtlich eines möglichen Einflusses auf die Thrombozytenfunktion und die Hämostase auf, da Serotonin ein zentraler Mediator der Thrombozytenaggregation und des Gefäßtonus ist. Während die Effekte von NSAR, Paracetamol und Metamizol auf die Thrombozytenfunktion gut dokumentiert sind, ist der Einfluss von Tramadol bisher unzureichend untersucht und uneinheitlich beschrieben. Frühere Studien berichten widersprüchliche Ergebnisse – von gesteigerter Aggregation bis zu verzögerter Gerinnselbildung – und unterstreichen somit die Notwendigkeit einer systematischen Untersuchung. Ziel dieser Studie war es, den Effekt von Tramadol in verschiedenen Konzentrationen auf die Thrombozytenfunktion zu analysieren, sowohl isoliert als auch in Kombination mit häufig gleichzeitig verabreichten Medikamenten wie Metamizol, Ibuprofen und Fentanyl.

Methoden

Diese monozentrische Laborstudie wurde an der Medizinischen Universität Graz nach Ethikvotum (IRB00002556) und Registrierung bei ClinicalTrials.gov (NCT05237492) durchgeführt. Dreiunddreißig gesunde erwachsene Probanden wurden nach Aufklärung und Einwilligung eingeschlossen. Ausschlusskriterien waren u. a. eine laufende Antikoagulation, Gerinnungsstörungen oder eine bestehende Opiattherapie. Aus den Blutproben wurde plättchenreiches Plasma gewonnen und mittels Lichttransmissionsaggregometrie (LTA), dem Referenzverfahren zur Untersuchung der Thrombozytenfunktion, analysiert. Tramadol wurde in Konzentrationen von 500 bis 9000 ng/ml titriert. Weitere Experimente testeten

Tramadol (200 ng/ml) in Kombination mit Metamizol (300–900 µg/ml), Ibuprofen (60–180 µg/ml) oder Fentanyl (3000–9000 ng/ml). Die Aggregation wurde durch Standard-Agonisten (ADP, Kollagen, Arachidonsäure, Ristocetin, TRAP) induziert. Für die statistische Auswertung wurden der Friedman-Test für nichtparametrische Messwiederholungen, Kendall's W zur Effektstärkenschätzung sowie Post-hoc-Vergleiche angewendet.

Ergebnisse

Die Ausgangswerte der Thrombozytenaggregation waren in den Kontrollgruppen konsistent. Tramadol allein führte selbst in supratherapeutischen Konzentrationen bei keinem der getesteten Agonisten zu statistisch signifikanten Veränderungen der Thrombozytenaggregation (ADP: p=0,284; Kollagen: p=0,382; TRAP: p=0,502; Ristocetin: p=0,404; Arachidonsäure: p=0,121). Die Effektstärken waren gering bis moderat.

Im Gegensatz dazu zeigte die Kombination von Tramadol mit Metamizol signifikante Reduktionen der Thrombozytenaggregation nach Aktivierung mit ADP (p=0,015), Kollagen (p=0,016) und Ristocetin (p=0,027), mit großen Effektstärken (Kendall's W bis zu 0,775). Ebenso führte Tramadol in Kombination mit Ibuprofen zu signifikanten hemmenden Effekten bei ADP- (p=0,021), Arachidonsäure- (p=0,013) und Kollagenaktivierung (p=0,038).

Für die Kombinationen von Tramadol mit Fentanyl wurden keine statistisch signifikanten Unterschiede beobachtet, allerdings zeigten sich in einigen Testverfahren Tendenzen zu einer verminderten Aggregation (p-Werte 0,058–0,126).

Conclusio

In dieser ex vivo-Studie zeigte Tramadol allein keinen signifikanten Einfluss auf die Thrombozytenaggregation in verschiedenen Signalwegen, was die Nullhypothese stützt. In Kombination mit Metamizol oder Ibuprofen hingegen war Tramadol mit einer Hemmung der Thrombozytenfunktion assoziiert, während mit Fentanyl keine Wechselwirkungen auftraten. Diese potenziellen Interaktionen sollten bei der perioperativen Schmerztherapie

berücksichtigt werden, insbesondere bei Patientinnen und Patienten mit erhöhtem Blutungsrisiko. Weitere klinische Studien mit größeren Fallzahlen sind erforderlich, um diese Ergebnisse zu bestätigen und ihre Übertragbarkeit in vivo zu prüfen.

Introduction

Background

In recent years, various analgesics have undergone investigation regarding their potential impact on platelet function or the coagulation cascade. Studies have demonstrated corresponding changes for paracetamol [1], NSAIDs like Ibuprofen [2], metamizole [3], as well as co-analgesics such as SSRIs [4], which have shown a notable increase in bleeding risk.

Interestingly, there is a scarcity of articles addressing the effect of tramadol on platelet function and blood coagulation.

Casella, S., et al. conducted a study examining the impact of tramadol on platelet aggregation in vitro using horse blood samples. The findings revealed a distinctive effect of tramadol varying between fasted and fed horses. Non-fasted horses exhibited a notably heightened increase in platelet aggregation, along with enhanced clot formation upon titration of tramadol. In contrast, fasted horses displayed no significant alterations in hemostasis compared to the control group. [5]

In contrast to the findings of Casella, S., et al., Bilir, A., et al. present opposing results in a human study. In this study, varying doses of tramadol are introduced to blood samples in vitro. As the tramadol dosage increases, there is a notable deceleration in clot formation observed in trombelastography (ROTEM). However, there are no differences in clot strength, as indicated by maximal clot formation (MCF), between the two groups. [6]

These conflicting examples underscore the necessity for further research in an area critical for patient safety, namely blood coagulation and platelet function in a perioperative setting.

The objective of this study is to examine the influence of tramadol on platelet function independently, across various doses, and in combination with other medications.

Tramadol

Tramadol ((±)-(1R*,2R*)-2-(Dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol) is one of the most commonly prescribed painkillers in Germany. It is structurally related to codeine and morphine and was first synthesized as an antitussive by the Grünenthal company (Stolberg) in 1962 and approved as an analgesic in 1977. [7]

Tramadol has a special pharmacodynamic profile: it not only binds to μ-receptors like other opioids , but also blocks the reuptake of serotonin and norepinephrine in the synaptic cleft. [8] It also has an influence on nicotinic and muscarinic receptors and potassium channels. [9-11]

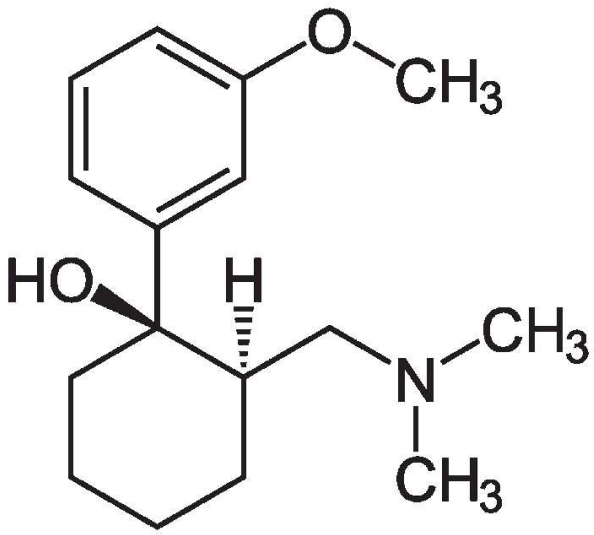

Figure 1: Structure of Tramadol ((±)-(1R*,2R*)-2-(Dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol), image in the public domain.

In addition, tramadol has modulatory effects on a variety of other mediators involved in pain mediation: glutamate recpetors, α_2-adrenoceptors, voltage-gated sodium ion channels, adenosine receptors and pathways involving calcitonin gene-related peptide, prostaglandin E_2, proinflammatory cytokines and substance P. Due to this large number of molecular and receptor targets, tramadol has a broad spectrum of activity: from analgesic effects on various types of pain such as neuropathic pain, postoperative pain, and pain associated with

labor, to antidepressant, anxiolytic, and antispasmodic effects [8], to the treatment of premature ejaculation. [12]

Its potency compared to morphine is only 0.05 - 0.09 so that it is administered in single doses of 100-150mg per administration and per person.

Like other opiates, tramadol blocks the serotonin transporter (SERT) and thus increases the extracellular serotonin concentration. In addition, the racemate and (+)-Tramadol acts as a releaser of serotonin. [13]

Tramadol is a recommended analgesic on the second step of the WHOs scheme on the treatment of pain [14] and according to the US drug spending report, it was among the twenty most prescribed drugs in the United States. [15]

Tramadol is generally well-tolerated and has a favorable side effect profile. The most common adverse effects include nausea (6.1%), dizziness (4.6%), drowsiness (2.4%), fatigue (2.3%), sweating (1.9%), vomiting (1.7%), and dry mouth (1.6%). [16]

Serotonin and its role in blood clotting

Serotonin (5-Hydroxytryptamin or 5-HT) is a neurotransmitter, its complex biological function is involved in multiple processes throughout the whole organism: from cognition and mood to memory, learning to vomitting. Serotonin was first described in the early 1930s by the Italian chemist and pharmacologist Vittorio Erspamer. Erspamer isolated Serotonin from the enterochromaffin cells of the intestine. For this reason, it was first referred to as entestamine. [17]

In addition to the numerous effects already mentioned, serotonin also has a direct influence on platelet aggregation. Large amounts of the serotonin made in the intestine are absorbed from the plasma and then stored in the blood platelets in dense granulates. [18]

As soon as it is released during platelet activation, serotonin acts as a mild platelet aggregator: Platelets carry serotonin receptors (5HT2), the activation of which leads to an increase in platelet aggregation triggered by other messenger substances such as adenosine diphosphate (ADP) and thrombin, it so co-operates with other proteins in a dose-dependent manner. [19, 20]

Although serotonin can have a vasodilatory effect, it also increases vasoconstriction in areas where the endothelium is damaged which is crucial for blood clotting as blood flow is reduced and clot formation is facilitated. [21, 22]

For this reason, drugs that influence the serotonin balance can also have an influence on platelet function and blood clotting. For example, there are already numerous studies on this for the group of selective serotonin reuptake inhibitors.

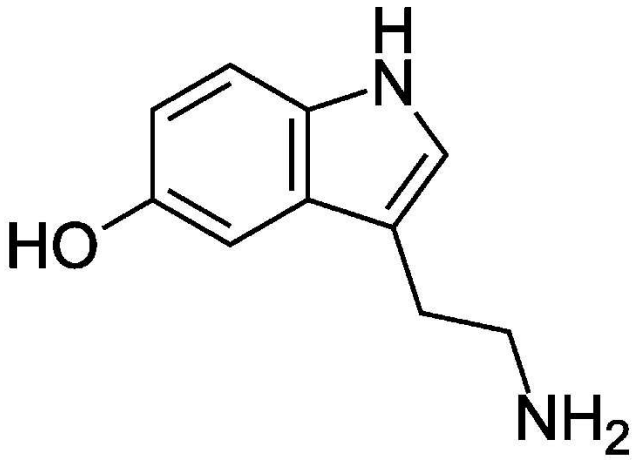

Figure 2: Structure of Serotonin (5-Hydroxytryptamin), image in the public domain.

Selective Serotonin Reuptake Inhibitors (SSRIs) and Risk of Bleeding

Numerous studies and case reports have investigated the risk of bleeding in people undergoing SSRI therapy. Many indicate an increased risk of bleeding, but not uniformly. The results depend not only on the dose and exact substance of the SSRI, but also on the location of the haemorrhage (e.g. GIT or cerebral haemorrhage).

In a meta-analysis of 42 observational studies (11 cohort studies with 187,956 subjects, 31 case-control studies with 1,255,073 patients), the risk of bleeding across all groups was increased in patients taking SSRIs compared to control subjects (OR 1.41, 95% CI 1.27-1.57). [23]

Gastrointestinal haemorrhage is the most commonly reported bleeding in patients taking SSRIs. Fifteen case-controlled studies with 393,268 participants and four cohort studies have shown that the number needed to harm (NNH) for upper gastrointestinal bleeding with SSRI treatment was between 881 and 3177. But the risk of bleeding increases significantly when patients are taking other medications known to affect platelets and other haemostatic mechanisms like NSAIDs, anticoagulants or antiplatelet drugs. [24-26]

Pain

Definitions

According to the IASP, the international Association for the study of Pain, pain is defined as follows:

„An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage.“ [27]

Pain is a complex sensory perception and normally has the function of an alarm signal whose intensity can range from unpleasant to unbearable. Chronic pain has lost the character of an alarm signal and is now regarded and treated as an independent clinical picture (chronic pain syndrome). The sensation of pain in itself is a sensory perception and sensory modality as a part of body perception (somatovisceral sensitivity).

Acute and chronic pain are not only medically highly relevant and complex diseases, but also of enormous economic importance. In the USA alone, costs of between 560 and 635 billion US dollars were estimated in 2011, which are made up of costs for sick leave, therapies and lower incomes of those affected and thus exceed the costs of cardiovascular diseases and cancer. [28]

WHO-Scheme

The WHO analgesic ladder was originally published in 1986 as a WHO guideline for pain management in patients who had cancer. It originally proposed a three-stage approach to pain management:

In the **first stage**, non-opioids are used. These are used up to a VAS of about 3-4 and have a so-called ceiling effect: when the dose administered is increased above the recommended maximum daily dose, there is no further increase in the effect, but rather an increase in side effects.

Active ingredients at this lowest level are:

- ASS
- Diclofenac
- Paracetamol
- Selective COX2-Inhibitors
- Ibuprofen and
- Metamizol

If the pain cannot be effectively controlled by means of the first stage of this scheme, weak opiods of the **second stage** should be added. These include:

- Tramadol and
- Dihydrocodeine

Both substances are pure μ-agonists and are available as sustained-release preparations to provide long-acting analgesia. Tramadol has about 10% of the potency of the reference substance morphine, dihydrocodeine about 20%.

In the **third stage** of the WHO's regimen, the weak-acting opioid is replaced by a strong one. There are a number of substances available, such as morphine as a reference substance or

Fentanyl and Buprenorphine, both of which are also available as transdermal systems. [29]

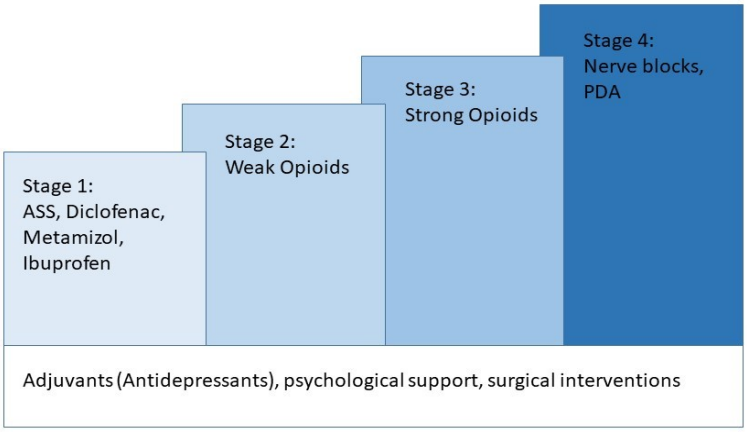

Figure 3: WHO-Scheme

The **fourth level** has been added in recent years and includes invasive techniques such as spinal and peridural injections, ganglion blocks and peripheral local anaesthesia.

Other Drugs used in this Study

Metamizole

Metamizole ([(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl)-methylamino]methanesulfonic acid) is the main representative of the pyrazolone derivatives, which include the older active substances phenazone, propyphenazone and phenylbutazone. It was first authorised in 1922 under the name "Novalgin". [30]

Figure 4: Structure of Metamizole ([(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl)-methylamino]methanesulfonic acid), image in the public domain.

The exact mechanism of action of metamizole is still unclear, an involvement of the opioid and 5HT metabolism appears possible, and metamizole also inhibits cyclooxygenase. [31] Other authors report that metamizole has a complex mechanism of action in which cannabinoid receptors could be addressed in addition to the inhibition of cyclooxygenase-3 and the activation of the opiod system. [32]

In 2015, it was shown to inhibit the „Transient Receptor Potential Ankyrin 1“ (TRPA1) ion channels in nociceptors. [33]

The active substance is expected to exert its effects at multiple levels. Furthermore, metamizole induces spasmolysis by opening potassium channels and diminishing the entry of calcium into smooth muscle cells. Apart from its analgesic properties, metamizole also possesses antipyretic effects.

Notable potential side effects of metamizole are drops in blood pressure, especially in hypovolaemic patients, which is caused by direct vasodilation. Anaphylactic reactions are rarer. An allergic agranulocytosis (type 2 reaction) occurs in approx. 5 out of 1 million cases of use; the occurrence is unpredictable and can also be caused by a single dose. It is caused by toxic bone marrow depression. Due to the potentially life-threatening agranulocytosis, Metamizol is no longer licensed in many countries.

Indications for the use of metamizole are limited to the following due to the unpredictable side effects, especially agranulocytosis:

- acute severe pain after surgery or trauma
- tumour-related pain
- pain due to colic
- fevers that cannot be reduced by other medications

It has been known for over four decades that metamizole inhibits platelet aggregation induced by ADP, collagen, epinephrine and arachidonic acid in vitro in a dose-dependent manner by inhibiting cyclooxygenase. [3, 34]

This effect sets in after only a few minutes following intravenous administration and lasts for up to 72 hours. [35]

Ibuprofen

Ibuprofen (2-(4-isobutylphenyl)propionic acid) is on the World Health Organisation's list of essential medicines and is a drug from the group of non-steroidal anti-inflammatory drugs (NSAIDs) that is used to treat pain, inflammation and fever.

Ibuprofen belongs to the group of arylpropionic acids, its name ibuprofen is derived from the chemical name of the substance 2-(4-isobutylphenyl)propionic acid. Ibuprofen works by non-selectively inhibiting the cyclooxygenases COX 1 and 2 so that the inflammation-mediating prostaglandins are reduced.

There are two isoforms of cyclooxygenase enzymes (COX): COX1, which is constitutively expressed, and COX2, which is inducible. These two enzymes are the main target for

ibuprofen and all other NSAIDs. COX enzymes are generally located in the endoplasmic reticulum, are membrane-bound and bifunctional and catalyse the conversion of arachidonic acid (AA) to prostaglandin G_2 (PGG_2) and in a second step to prostaglandin H_2 (PGH_2). [36]

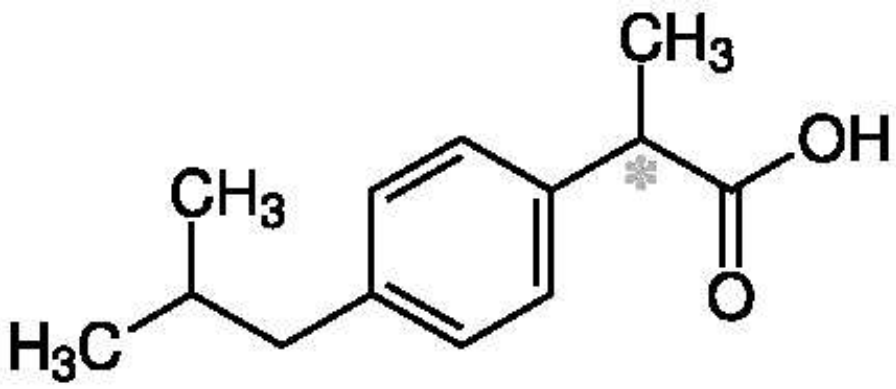

Figure 5: Structure of ibuprofen (2-(4-isobutylphenyl)propionic acid), image in the public domain.

The enzyme thromboxane synthase (TXAS), which is mainly found in thrombocytes, catalyses the conversion of prostaglandin H_2 to thromboxane A_2. Thromboxane promotes the constriction of blood vessels and is the natural antagonist of prostacyclin and is the main prostaglandin produced there. TXAS is also found in the lungs, kidneys, spleen and macrophages.

Thromboxan activates platelet aggregation via the thromboxane receptors on the platelets. Thromboxan triggers platelet aggregation by binding to thromboxane receptors located on the platelets. These platelets, or thrombocytes, exclusively contain cyclooxygenase-1 for prostaglandin synthesis, a process rendered permanently dysfunctional by acetylsalicylic acid. Consequently, prostaglandin formation cannot be resumed within platelets due to their lack of a nucleus. This impairment significantly limits the platelets' ability to aggregate for the duration of their lifespan in the body, which typically ranges from eight to twelve days, even following low doses of acetylsalicylic acid.

Contrary to aspirin, the inhibition caused by S-ibuprofen is temporary because it binds reversibly to COX-1 and last 4 to 6 hours. [37]

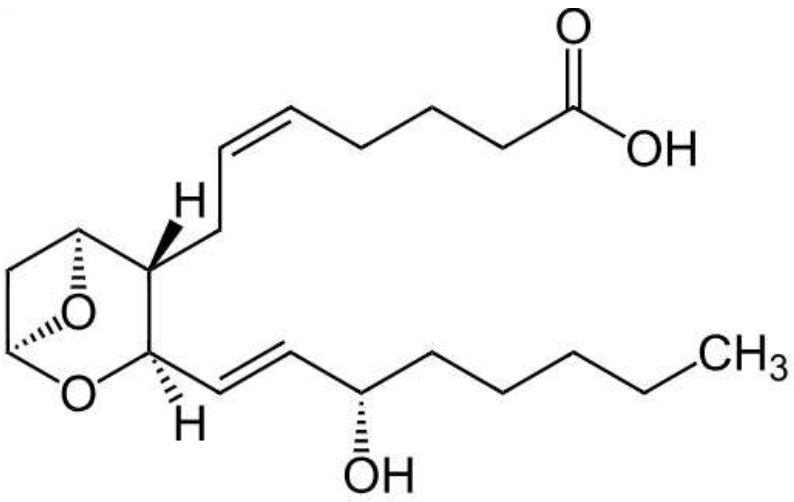

Figure 6: Structure of Thromboxan A2, image in the public domain.

It's noteworthy that combining ibuprofen with SSRIs can elevate the risk of gastrointestinal bleeding. This is because thrombocytes are unable to uptake serotonin, crucial for their function.

Fentanyl

Fentanyl (N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]propanamide) is a synthetic opioid and acts as a full agonist at the μ-opioid receptor Fentanyl has approximately 100 times the potency of the reference substance morphine is employed in the management of severe acute and chronic pain across various medical fields including anesthesia, intensive care, emergency medicine, and outpatient pain therapy. It is administered via injection, nasal spray, plaster, and absorption through the oral mucosa.

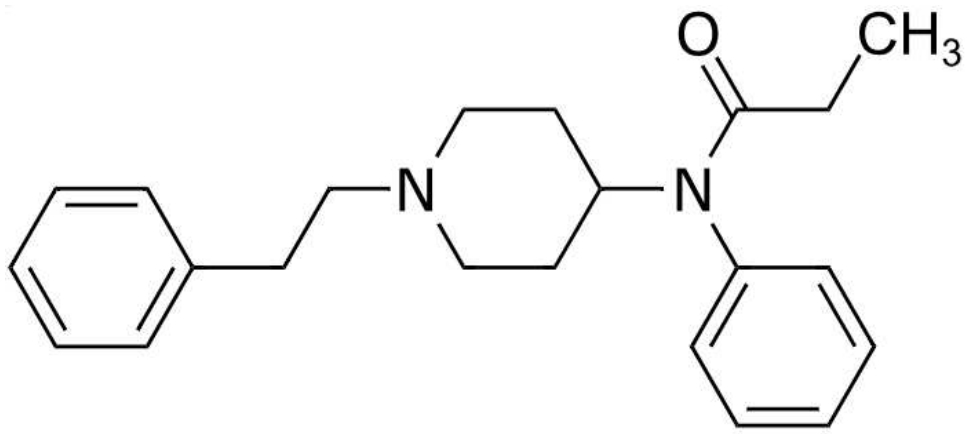

Figure 7: Structure of Fentanyl (N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]propanamide), image in the public domain.

In 1960, Paul Janssen was able to produce fentanyl for the first time in an attempt to improve cerebral transit by increasing the lipophilicity of the existing synthetic opiates phenoperidine and dextromoramide, which was first authorised in 1963.

The serotonin transporter (SERT, also 5-hydroxytryptamine transporter 5-HTT) is found in both platelets and nerves and its task is to maintain low serotonin levels in the plasma. One of its main tasks is to remove the released serotonin in the synaptic cleft and transport it into the cell interior, thus ending the serotonin effect, the cell-inward transport is driven by the sodium/potassium ATPase, serotonin thus reaches the cell interior in exchange with potassium. [38]

Despite this effect on the serotonin transporter, fentanyl has no effect on platelet function under various experimental settings. [39]

Fentanyl is one of the six clinically most used WHO step 3 opioids along with buprenorphine, hydromorphone, methadone, morphine, oxycodone. [40]

Light transmission aggregometry

The reference and standard method for measuring platelet function is Light Transmission Aggregometry (LTA) and has been established in clinical routine for 50 years. It was developed by Born and O'Brien and the principle of the test appears quite simple: platelet-rich plasma (PRP) which is produced by centrifuging blood appears cloudy due to the platelets and therefore allows little light transmission. This PRP is placed between a light source and a photocell. By adding agonists, the platelets aggregate, the PRP becomes clearer and light transmission increases. [41, 42]

Possible Agonists are:

- adenosine diphosphate (ADP)
- arachidonic acid
- collagen
- ristocetin
- Thrombine activating peptide (TRAP)
- epinephrine or
- thromboxane

The choice of agonist depends on the clinical suspicion and the aim of the investigation:

- **Initial screening:** ADP and collagen are often used as first choice agonists for general screening.
- **Specific disorders:** Depending on the suspected disorder, specific agonists may be chosen (e.g. arachidonic acid for aspirin resistance or ristocetin for he diagnosis of the vanWillebrand-Syndrom or the Bernard-Soulier-syndrom).
- **Combination tests:** The use of several agonists enables a more comprehensive assessment of platelet function. [43, 44]

	ADP	Epinephrine	Collagen	Ristocetin
P2Y receptor inhibitor or defect	**Decreased**	Normal	Normal	Normal
Adrenergic recetor defect	Normal	**Decreased**	Normal	Normal
Collagen receptor defect	Normal	Normal	**Decreased or absent**	Normal
Von Willebrand disease	Normal	Normal	Normal	**Decreased or absent**
Bernard-Soulier syndrome	Normal	Normal	Normal	**Decreased or absent**
Glanzmann´s thrombasthenia	**Decreased**	**Decreased**	**Decreased**	**Normal**

Table 1: Overview of possible combinations various coagulation disorders in the LTA. [45]

Activation causes the glycoprotein GP IIb/IIIa to be formed on the surface of the platelets. This allows fibrinogen to bind to GP IIb/IIIa and bind so many platelets together that platelet aggregates are formed.

Possible influencing factors that can lead to aggregation through activation of the thrombocytes and thus falsify the result are

- Cold stored samples
- Shear stress during blood collection, e.g. due to cannulas that are too small
- Too long storage time as the platelets quickly lose their function outside the body
- Too few platelets, e.g. due to excessive centrifugation [46]

As the LTA is a routine test in the laboratory of the Clinical Institute for Medical and Chemical Laboratory Diagnostics, these possible influencing factors could already be excluded in the design phase of this study.

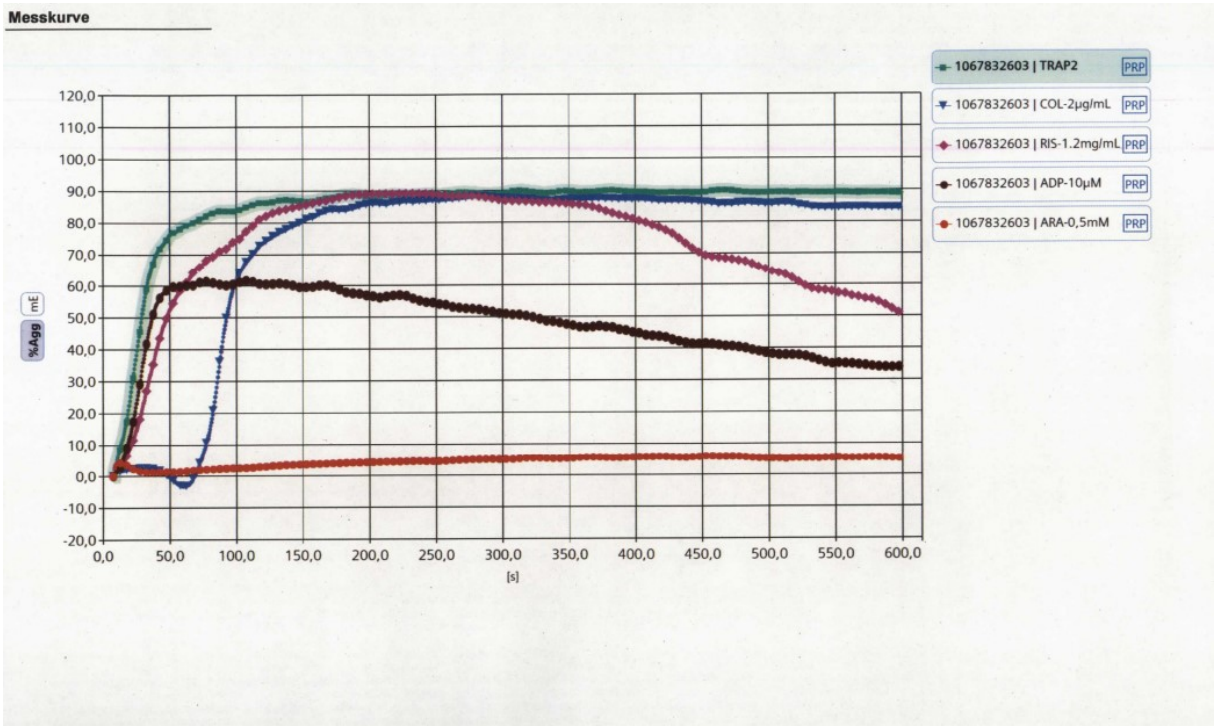

Figure 8: Example of LTA result after the addition of tramadol and metamizole

Aims and hypothesis

Theoretical framework

The starting point for the main idea and hypothesis and the theoretical framework of this study are the following considerations: Tramadol has an effect on serontonin levels in the synaptic cleft by acting as a serotinin reuptake inhibitor. Serotonin has multiple effects throughout the human organism and plays an important role in blood clotting by acting as a mild platelet aggregator and vasoconstrictor.

If more serotonin is present in the synaptic cleft or plasma due to the blocking of serotonin reuptake by tramadol, we expect tramadol to have an effect on platelet function that may be enhanced in combination with other drugs that act in this area and are known to have an effect on coagulation and platelet function.

Research question 1

The primary aim of this study was to quantify the effect of tramadol on platelet aggregation. For this purpose, the effect of tramadol on platelet function is analysed in an ex-vivo study using light-transmission aggregometry.

The main objective is to determine this influence on thrombocyte function by means of thrombocyte function test.

Research question 2

In addition, tramadol is often administered together with other medications such as NSAIDs like ibuprofen, novalgin or other opioids such as morphine or fentanyl in the operative setting. An additional series of studies is therefore investigating whether these combinations alter the influence of tramadol on platelet function.

Null hypothesis

The null hypothesis of this study is that tramadol does not affect platelet aggregation in healthy patients.

Methods

General information

Parts of the data and information presented here have already been published in *Haemostaseologie* in press). [47] These results form part of my dissertation and are reproduced here in part, with partially identical content.

Registration and Institutional Review board

In a first step, the study was planned in accordance with good scientific practice and written down in a study protocol. The study was then approved by the Institutional Review Board of the Medical University of Graz (33-553 ex 20/21; Ethics Committee of the Medical University of Graz, IRB00002556, Chairman Prof. Josef Haas) on 30 September 2021 for one year before the start of patient recruitment, and this vote was extended for another year in September 2022.

The study was registered on ClinicalTrials.gov under the registration number ClinicalTrials.gov Identifier NCT05237492 on February 14th, 2022.

Study design

This study was designed as a single-center laboratory study according to the study protocol.

Sample size calculation

To plan our required samples, we calculated the number of cases using the following values:

- Expected effect of 25%
- Power 0.8, alpha 0.05
- Mean value of the AUC of the LTA using ADP 620.3; SD 40.5
- Mean difference: 60.5

This would result in a sufficient number of cases of 7. However, in order to generate a sufficient number of blood samples for our further questions and similar studies on other pain medications (e.g. Munsterhjelm et al. 2005 on paracetamol and its effects on thrombocyte function or Martini et al. 2016 on the influence of Ibuprofen on platelet aggregation) that used 6 - 15 subjects, we also orientated ourselves on these numbers. [1, 48]

The initial plan was to perform all tests on these 15 samples, but as we realised during the first tests that we could not generate enough PRP from the samples to perform all test series, our plan was changed as follows:

- 15 samples with Tramadol only, then
- 6 samples each with ibuprofen, metamizole and fentanyl in increasing titration series

Study setting

This study was performed at the Department of Anaesthesiology and Intensive Care Medicine (Division of General Anaesthesiology and Intensive Care Medicine 1) of the Medical University of Graz. The laboratory tests (LTA) were carried out at the Clinical Institute for Medical and Chemical Laboratory Diagnostics (KIMCL) at the LKH University Hospital Graz during routine work hours.

According to the study protocol, a total of 33 patients who were examined in the preoperative anaesthesia outpatient clinic at the LKH University Hospital were included in this study.

Recruitment of participants

Inclusion criteria

Adults aged 18 years and older, who volunteered for this study.

Exclusion criteria

1. Age < 18 years
2. Pregnant women
3. History of addiction (especially opiate abuse)
4. Pre-existing general addictive disease
5. Ongoing pain therapy with opiates
6. Taking antidepressants (SNRI, SSRI)
7. History of thrombocytopathy or coagulation disorders
8. Therapy with drugs that influence thrombocyte function (ASS, clopidogrel, prasugrel, ticagrelor or similar)
9. Known intolerance to opiates

Informed consent

When potential participants of our study agreed to participate in the anaesthesia outpatient clinic, they were first informed comprehensively and according to the contents of our informed consent form about possible side effects and the procedure. Once this informed consent was given, they were screened for exclusion criteria by means of a questionnaire.

Specimen materials

After the informed consent and the questionnaire were completed and signed, 10 tubes of blood were taken from each patient in accordance with the study protocol using Greiner Bio One VACUETTE® citrate tubes 3,5ml according to the hospital standard in the anaesthesia outpatient department by a nurse and taken directly and without delay to the local

laboratory by the head of the study. Afterwards, the participants were observed for about 10 minutes for potential side effects such as circulatory problems.

Laboratory methods

After centrifugation, a baseline platelet function was measured with platelet-rich Plasma (PRP), then the drug according to our study protocol were added in increasing concentrations. Following drugs and dosages were tested according to the respective research question:

Research question 1

- Tramadol (therapeutic plasma level: 100 – 1000 ng/ml) [49] with
  - 500 ng/ml,
  - 1500 ng/ml,
  - 4500 ng/ml and
  - 9000 ng/ml

Research question 2

- Tramadol (200ng/ ml) in combination with Metamizole (therapeutic plasma level: 10 µg/ ml) [49] with
  - 300 µg/ ml,
  - 600 µg/ ml and
  - 900 µg/ ml

- Tramadol (200ng/ ml) in combination with Fentanyl (therapeutic plasma level: 0,3 – 300 ng/ ml) [49] with
  - 3000 ng/ ml,
  - 6000 ng/ ml and
  - 9000 ng/ ml

- Tramadol (200ng/ ml) in combination with Ibuprofen (therapeutic plasma level: 15-30 µg/ ml) [49] with
  - 60 µg/ ml,

- 120 µg/ ml and
- 180 µg/ ml

We used supratherapeutic concentrations to ensure detectable pharmacodynamic effects under in vitro conditions, where dilution constraints inherent to the experimental setup limited the achievable concentration ranges. These concentrations were selected based on preliminary concentration-finding experiments, in which lower concentrations did not produce measurable effects on platelet aggregation under the applied in vitro conditions.

Light transmission was measured after the addition of different agonists and the result was described as the percentage of maximum aggregation. The following agonists were used in our setting, only results of the highest concentratins of agonists were used for our calculations:

- ADP (5 + 10µM)
- arachidonic acid (0,5 + 1 mM)
- collagen (1 + 2µg/ ml)
- TRAP (50µM)
- Ristocetin (0,6 + 1,2mg /l)

The measurement procedure was performed according to the manufacturer's instructions.

Originally, the study protocol also planned to carry out TEG investigations in accordance with the concentration series. However, due to the small amount of blood and the capacities in the labor, this was cancelled at the beginning of the study.

All measurements were carried out within 4 hours after blood collection.

Standardized platelet function testing was performed by optical aggregometry on the Atellica COAG 360 coagulation analyser with an integrated four channel Aggregometer (Siemens-Healthineers, Vienna, Austria).

Statistical methods and analysis

The data collected was pseudonymised and collected in a Spreadsheet (.XLSX-format). After completion of the transferred to data collection phase, data were transferred to a database in the statistics and analysis software SPSS (IBM SPSS Statistics 27) for further analysis.

General and epidemiological data such as age, gender and medication taken were taken from the questionnaire and analysed descriptively and statistically in Microsoft Excel (Microsoft, Redmont, USA).

The statistical analyses were conducted descriptively and statistically.

We calculated the percentage difference from baseline for each subject. Data was not normally distributed so Friedman´s-Test for non-normal distributed indipendent samples was used, Kendall´s W was calculated to quantify the magnitude of the observed effects and guide estimation of sample sizes for future studies. Acoording to Cohen, $W < 0,1$ would be a small effect size and $>0,5$ would be a large effect size. [50]

In addition, an individual case analysis was performed.

Results

Parts of the data and information presented here have already been published in *Haemostaseologie* in press). [47] These results form part of my dissertation and are reproduced here in part, with partially identical content.

General patients characteristics

In total, 33 voluntary participants could be recruited for this study, no one had to be excluded after completing the questionnaire.

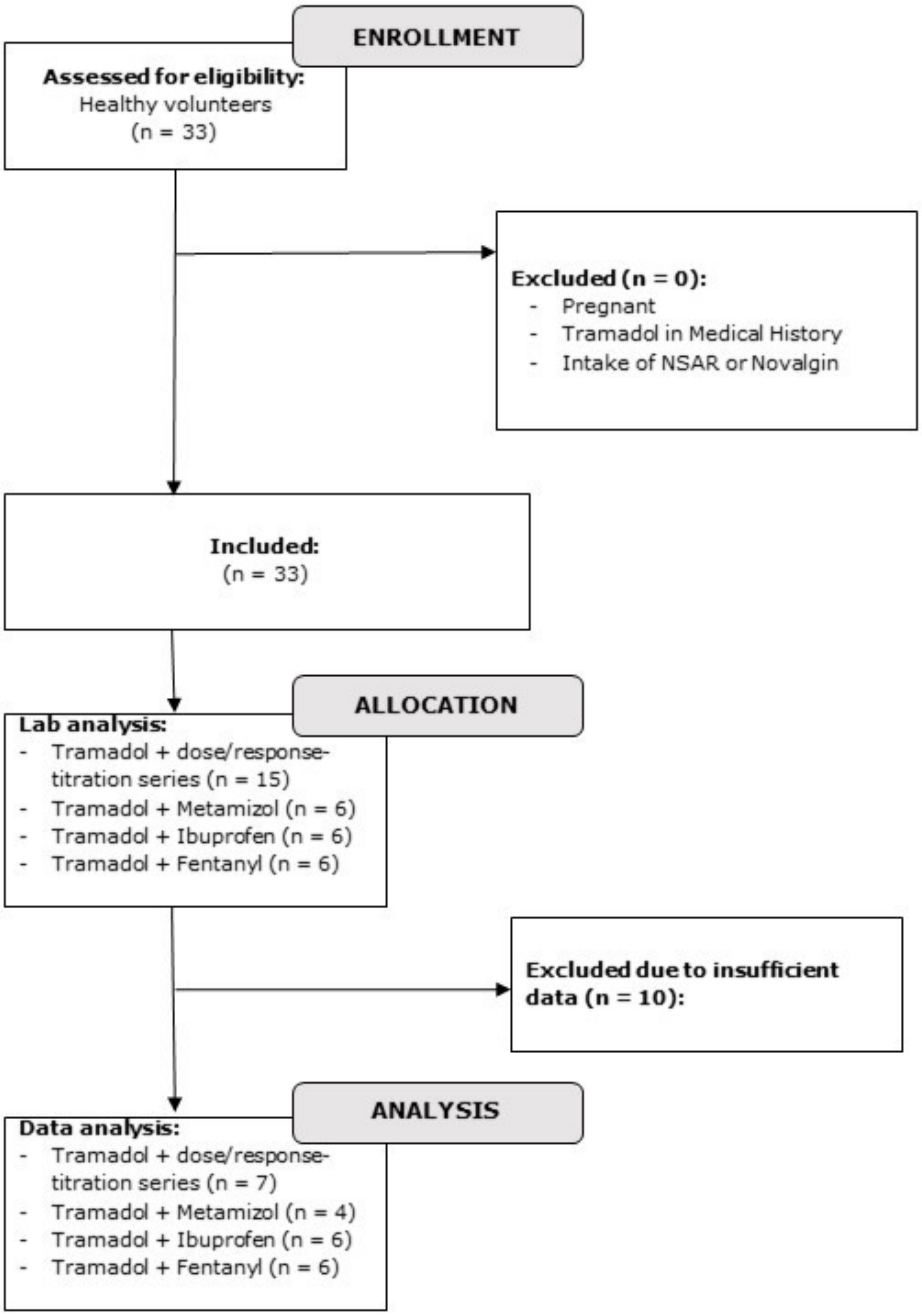

Figure 9: Study Flow Chart

The median age of the participants was 36 years (27,67 - 36,59), 24% of them were female. Two of the 33 participants were frequent smoker (6%).

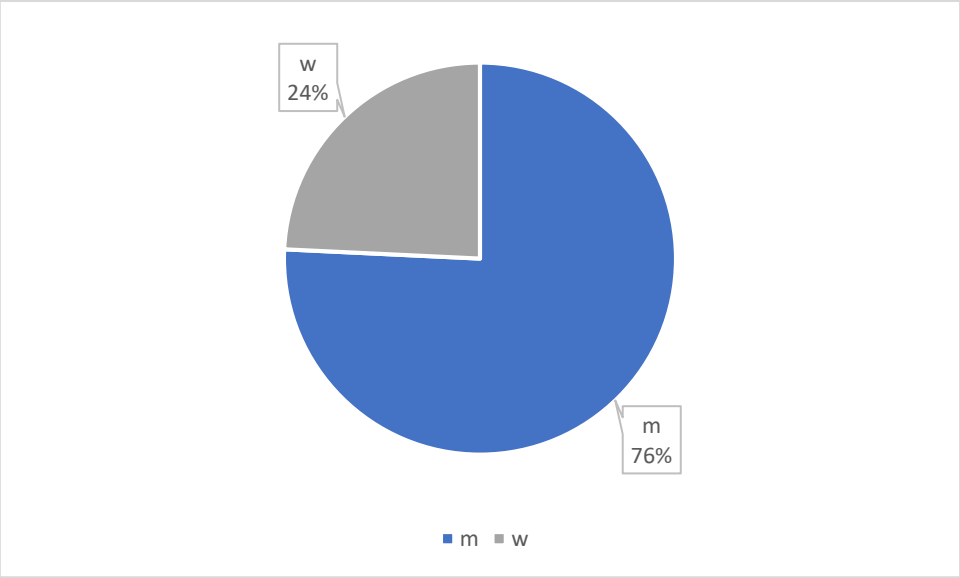

Figure 10: Shares in percent female/ male

General Information

The data was tested for normal distribution using the Kolmorogov-Smirnov test and visual inspectation as the number of subjects was relatively small at each group. As not all results are normally distributed ($p < 0.05$), no normal distribution was assumed.

Since aliquots were formed from each blood sample and each was tested at a specific concentration, we considered the results to be connected. Friedman test was used for the statistical analysis of non-normally distributed, connected samples.

To quantify the magnitude of the observed effects and guide estimation of sample sizes for future studies, Kendall's W was calculated. Due to the exploratory nature of this analysis, no correction for multiple testing was made.

In addition, an individual case analysis was performed.

Tramadol

ADP-Activation (10µM)

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
3	80,49	51,03	44,07	45,36	85,81	88,95
4	94,04	84,69	91,38			93,6
7	83,72	81		79,6		77,55
8	87,2	87,63		90,28		87,05
9	93,36	90,52	88,44	89,69	92,04	87,59
10	93,22	90,05	88,93	88,47	82,41	92,67
13	86,62	81,87	75,53	65,31		88,16
Q1	85,17	81,44	75,53	68,88	84,11	87,32
Median	87,20	84,69	88,44	84,04	85,81	88,16
Q3	93,29	88,84	88,93	89,39	88,93	90,81

Table 2: LTA-Results after addition of Tramadol in rising concentrations, ADP-activation

In this test, ADP was used as an agonist at a concentration of 10 µM. The baseline thrombocyte function, without the addition of Tramadol, was 87,20% (85,17% – 93,29%). Adding Tramadol at increasing concentrations resulted in the following median thrombocyte function levels:

- **500 ng/ml:** 84,69% (81,44% - 88,84%)
- **1500 ng/ml:** 88,44% (75,53% - 88,93%)
- **4500 ng/ml:** 84,04% (68,88% - 89,39%)
- **9000 ng/ml:** 85,81% (84,11% - 88,93%)

Baseline 2 without Tramadol showed a median thrombocyte function of 88,16% (87,32% - 90,81%).

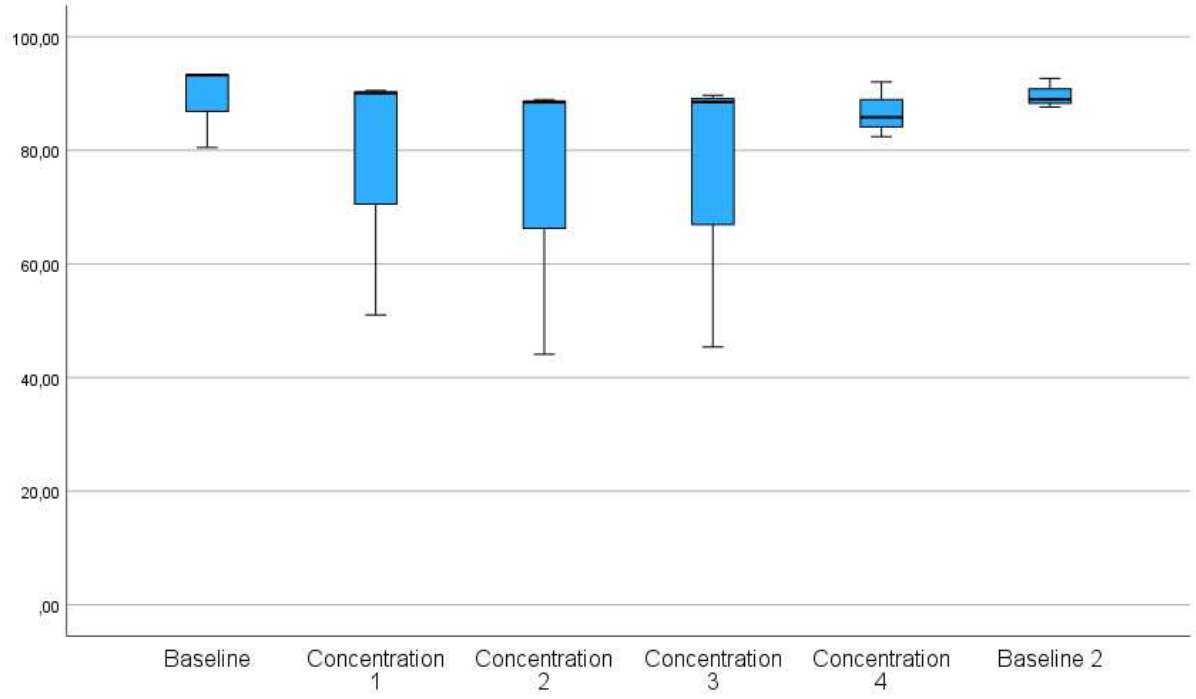

Figure 11: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 10µM ADP

There are no significant differences between the groups (p=0,284). Kendall's W for ADP was 0,416.

Arachidonic-Acid-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
3	87,38	5,37	7,35	4,62	3,03	89,17
4	94,49	1,49	83,87			96,00
7	2,51	1,44		2,23		3,41
8	88,35	89,81		92,15		88,15
9	88,93	92,70	89,52	88,28	7,95	87,92
10	94,81	95,00	88,70	90,32	84,65	88,92
13	85,86	2,22	5,82	3,55		87,29
Q1	86,62	1,86	7,35	3,82	5,49	87,61
Median	88,35	5,37	83,87	46,45	7,95	88,15
Q3	91,71	91,26	88,70	89,81	46,30	89,05

Table 3: LTA-Results after addition of Tramadol in rising concentrations, ARA-activation

In this test, arachidonic acid was used as agonist at a concentration of 1mM. The baseline thrombocyte function, without the addition of Tramadol, was 88,35% (86,62% - 91,71%). Adding Tramadol at increasing concentrations resulted in the following median thrombocyte function levels:

- **500 ng/ml:** 5,37% (1,86% - 91,26%)
- **1500 ng/ml:** 83,87% (7,35% - 88,70)
- **4500 ng/ml:** 46,45% (3,82% - 89,81%)
- **9000 ng/ml:** 7,95% (5,49% - 46,30%)

The 2nd control group without Tramadol showed a median thrombocyte function of 88,15% (87,61% - 89,05%).

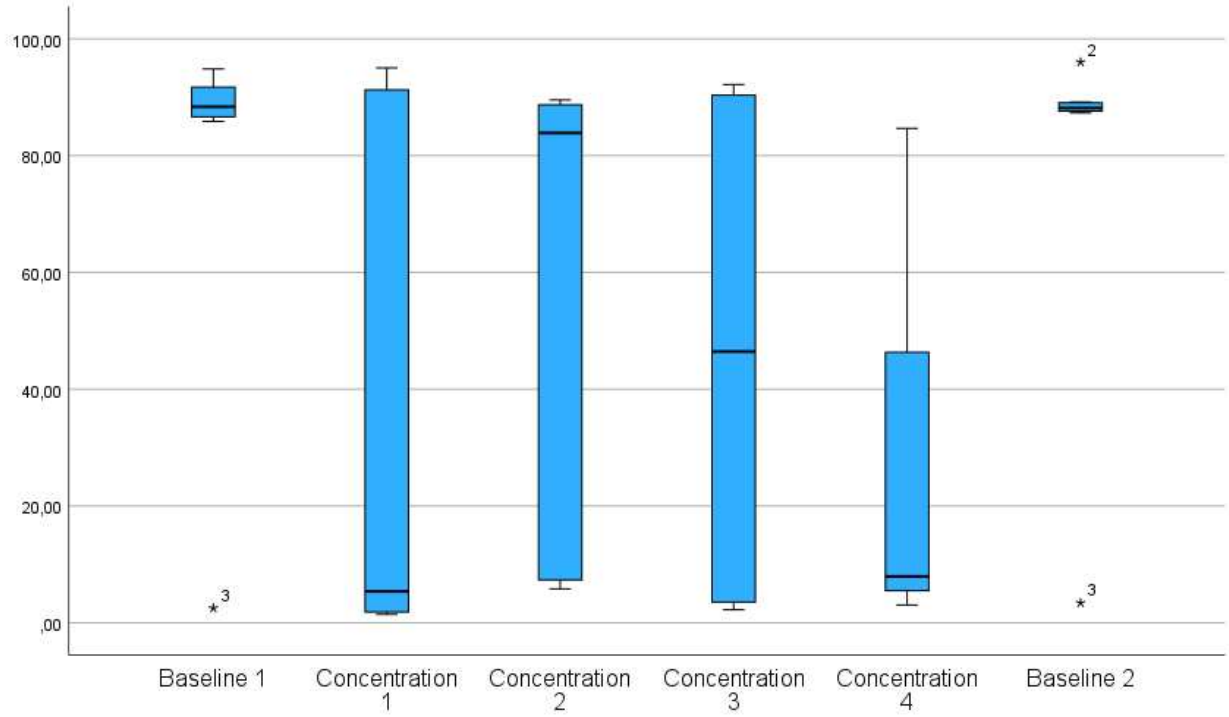

Figure 12: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 1mM arachidonic acid

There are no significant differences between the groups (p=0,121). Kendall's W for arachidonic acid was 0,581.

Thrombin receptor activating petide (TRAP) - Activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
3	88,33	90,06	82,72	82,67	91,34	91,4
4	88,42	92,94	91,04			89,05
7	89,95	90,26		90,52		88,92
8	88,93	89,45		89,6		86,89
9	84,16	88,19	88,75	88,46	90,36	92,63
10	89,83	94,6	89,22	93,78	89,44	89,69
13	87,99	88,52	89,52	87,21		87,31
Q1	88,16	88,99	88,75	87,52	89,9	88,12
Median	88,42	90,06	89,22	89,03	90,36	89,05
Q3	89,38	91,6	89,52	90,29	90,85	90,55

Table 4: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation, TRAP-activation

In this test, TRAP was used as agonist at a concentration of 50µM. The baseline thrombocyte function, without the addition of Tramadol, was 88,42% (88,16% - 89,38%). Adding Tramadol at increasing concentrations resulted in the following median thrombocyte function levels:

- **500 ng/ml:** 90,06% (88,99% - 91,06%)
- **1500 ng/ml:** 89,22% (88,75% - 89,52%)
- **4500 ng/ml:** 89,03% (87,52% - 90,29%)
- **9000 ng/ml:** 90,36% (89,90% - 90,85%)

The 2nd control group without Tramadol showed a median thrombocyte function of 89,05% (88,12% - 90,55%).

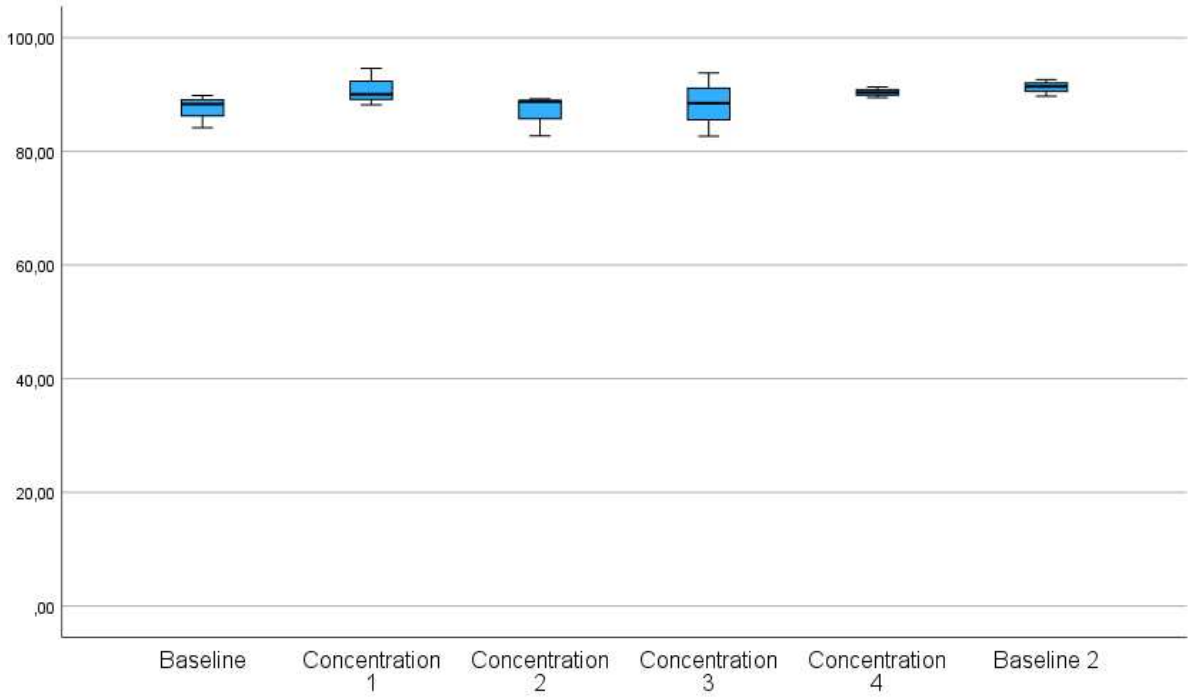

Figure 13: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation, using activation with 50µM TRAP

There are no significant differences between the groups (p=0,502). Kendall's W for TRAP was 0,289.

Ristocetin-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
3	91,04	90,77	88,54	86,28	87,21	89,28
4	94,21	55,24	88,43			92,71
7	81,63	88,23		88,82		85,05
8	88,13	89,63		88,68		91,52
9	91,26	83,24	90,96	90,86	89,29	92,86
10	89,58	95,9	89,87	91,09	88,73	90,47
13	87,25	83,08	78,09	67,96		85,52
Q1	87,69	83,16	88,43	86,88	87,97	87,40
Median	89,58	88,23	88,54	88,75	88,73	90,47
Q3	91,15	90,20	89,87	90,35	89,01	92,12

Table 5: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation, Ristocetin-activation

In this test, Ristocetin was used as the agonist at a concentration of 1,2mg /ml. The baseline thrombocyte function, without the addition of Tramadol, was 89,58% (87,69% - 91,15%). Adding Tramadol at increasing concentrations resulted in the following median thrombocyte function levels:

- **500 ng/ml:** 88,23% (83,16% - 90,20%)
- **1500 ng/ml:** 88,54% (88,43% - 89,87%)
- **4500 ng/ml:** 88,75% (86,88% - 90,35%)
- **9000 ng/ml:** 88,73% (87,97% - 89,01%)

The 2nd control group without Tramadol showed a median thrombocyte function of 90,47% (87,40% - 92,12%).

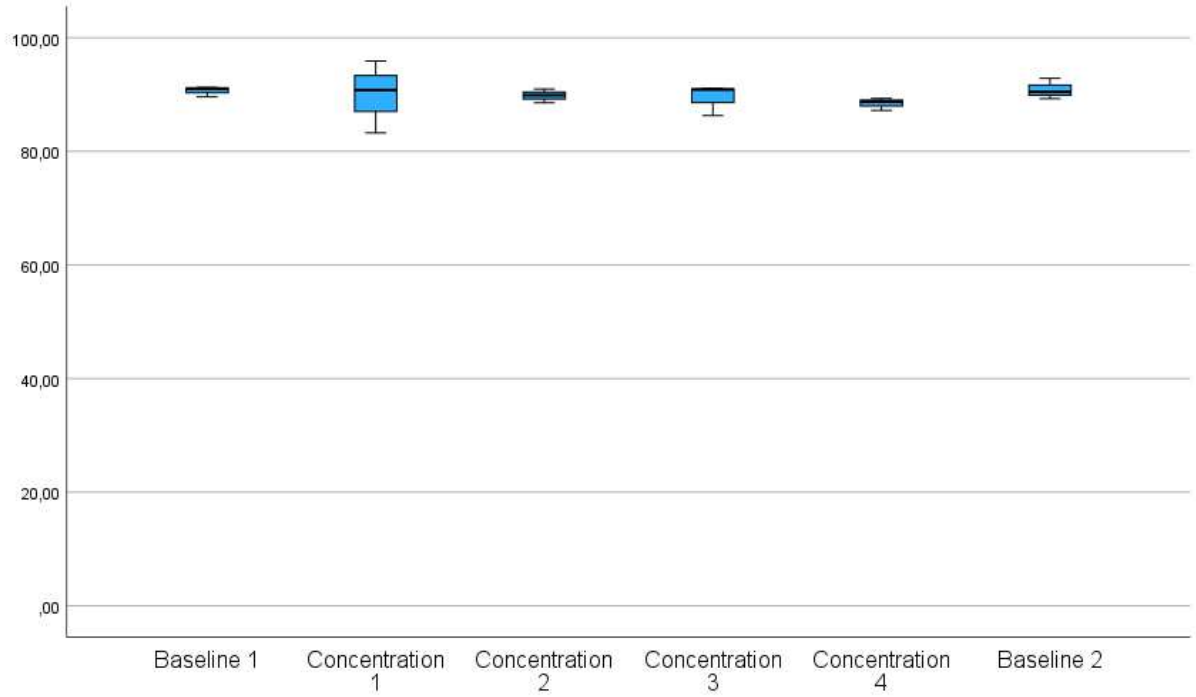

Figure 14: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with Ristocetin 1,2mg/ ml

There are no significant differences between the groups (p=0,404). Kendall's W for Ristocetin was 0,340.

Collagen-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
3	87,24	90,85	54,03	28,4	85,63	87,71
4	93,73	89,4	93,71			92,87
7	88,57	89,26		88,46		87,44
8	88,55	86,8		89,7		86,1
9	83,9	93,06	87,87	88,43	89,57	84,59
10	93,8	93,64	91,19	91,59	88,1	90,5
13	89,82	88,85	75,87	63,04		88,24
Q1	87,90	89,01	75,87	69,39	86,87	86,77
Median	88,57	89,40	87,87	88,45	88,10	87,71
Q3	91,78	91,96	91,19	89,39	88,84	89,37

Table 6: LTA-Results after addition of Tramadol in rising concentrations and collagen

In this test, collagen was used as agonist at a concentration of 2µg/ ml. The baseline thrombocyte function, without the addition of Tramadol, was 88,57% (87,90% – 91,78%). Adding Tramadol at increasing concentrations resulted in the following median thrombocyte function levels:

- **500 ng/ml:** 89,40% (89,01% - 91,96%)
- **1500 ng/ml:** 87,87% (75,87% - 91,19%)
- **4500 ng/ml:** 88,45% (69,39% - 89,39%)
- **9000 ng/ml:** 88,10% (86,87% - 88,84%)

The 2nd control group without Tramadol showed a median thrombocyte function of 87,71% (86,77% - 89,37%).

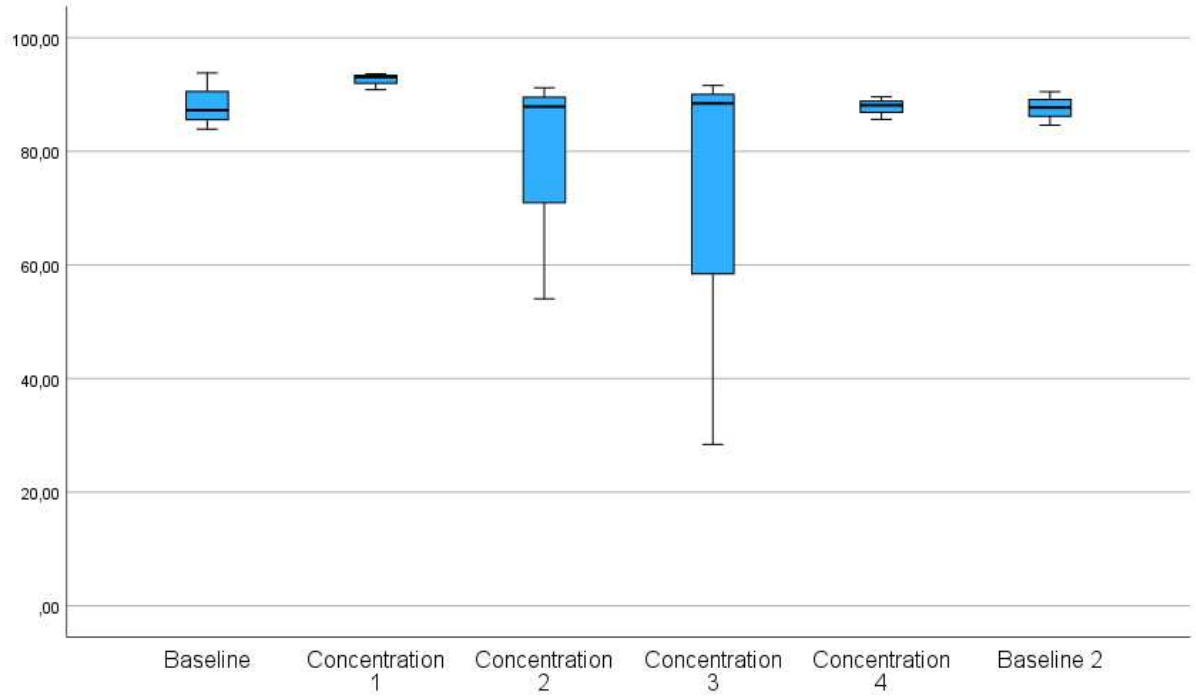

Figure 15: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with collagen 2µg/ ml

A statistically significant difference in thrombocyte function was not observed between the different concetration groups with collagen-activation (p=0,382). Kendall´s W for collagen was 0,352.

Tramadol in combination with other relevant drugs

Metamizol

ADP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	92,06	61,58	64,49	64,82	86,83
2	77,96	73,2	63,24	67,91	80,49
3	88,95	51,03	44,07	45,36	85,81
6	89,32	87,1	83,08	85,2	86,17
Q1	86,20	58,94	58,45	59,96	84,48
Median	89,14	67,39	63,87	66,37	85,99
Q3	90,01	76,68	69,14	72,23	86,34

Table 7: LTA-Results after addition of Tramadol and Metamizol in rising concentrations and ADP

In this test, ADP was used as an agonist at a concentration of 10 µM. The baseline thrombocyte function, without the addition of tramadol, was 89,14% (86,20% – 90,01%). Adding tramadol (200ng/ ml) and metamizol at increasing concentrations (300 µg/ ml, 600 µg/ ml and 900 µg/ ml) resulted in the following median thrombocyte function levels:

- **300 µg/ ml:** 67,39% (58,94% - 76,68%)
- **600 µg/ ml:** 63,87% (58,45% - 69,14%)
- **900 µg/ ml:** 66,37% (59,96% - 72,23%)

The 2nd control group without addition of tramadol and metamizol showed a median thrombocyte function of 85,99% (84,48% - 86,34%).

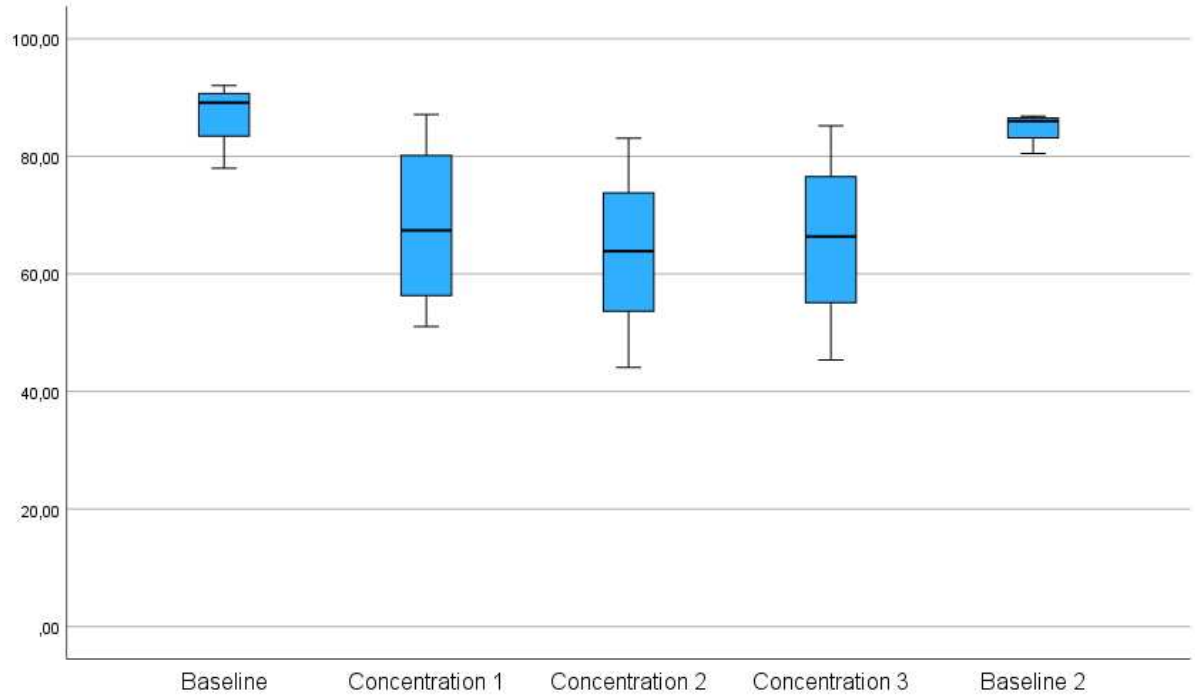

Figure 16: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 10µM ADP

A statistically significant difference in thrombocyte function after ADP-activation and addition of tramadol in combination with metamizol was observed between the different concetration groups, p=0,015. A post-hoc analysis between the different groups was performed and a significant difference between Baseline and Concetration 2 (p=0,017) was detected.

Kendall´s W was 0,775.

Arachidonic-Acid-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,5	5,94	3,05	9,16	1,86
2	81,01	2,29	2,86	0	87,38
3	89,17	5,37	7,35	4,62	3,03
6	87,49	88,7	86,76	88,7	88,09
Q1	85,87	4,60	3,00	3,47	2,74
Median	88,33	5,66	5,20	6,89	45,21
Q3	89,75	26,63	27,20	29,05	87,56

Table 8: LTA-Results after addition of Tramadol and Metamizol in rising concentrations and arachidonic acid

In this test, arachidonic acid was used as the agonist to assess platelet function at a concentration of 1mM. The baseline platelet function, measured without tramadol, was 88,33% (85,87% – 89,75%). When tramadol (200 ng/ml) was added along with increasing concentrations of metamizol (300 µg/ml, 600 µg/ml, and 900 µg/ml), the following median platelet function values were recorded:

- **300 µg/ml metamizol:** 5,66% (4,60% – 26,63%)
- **600 µg/ml metamizol:** 5,20% (3,00% – 27,20%)
- **900 µg/ml metamizol:** 6,89% (3,47% – 29,05%)

The second control group, which did not receive tramadol or metamizol, showed a median platelet function of 45,21% (2,74% – 87,56%).

A statistically significant difference in thrombocyte function after arachidonica-acid-activation and addition of tramadol in combination with metamizol was not observed between the different concetration groups (p=0,648), Kendall´s W was 0,155.

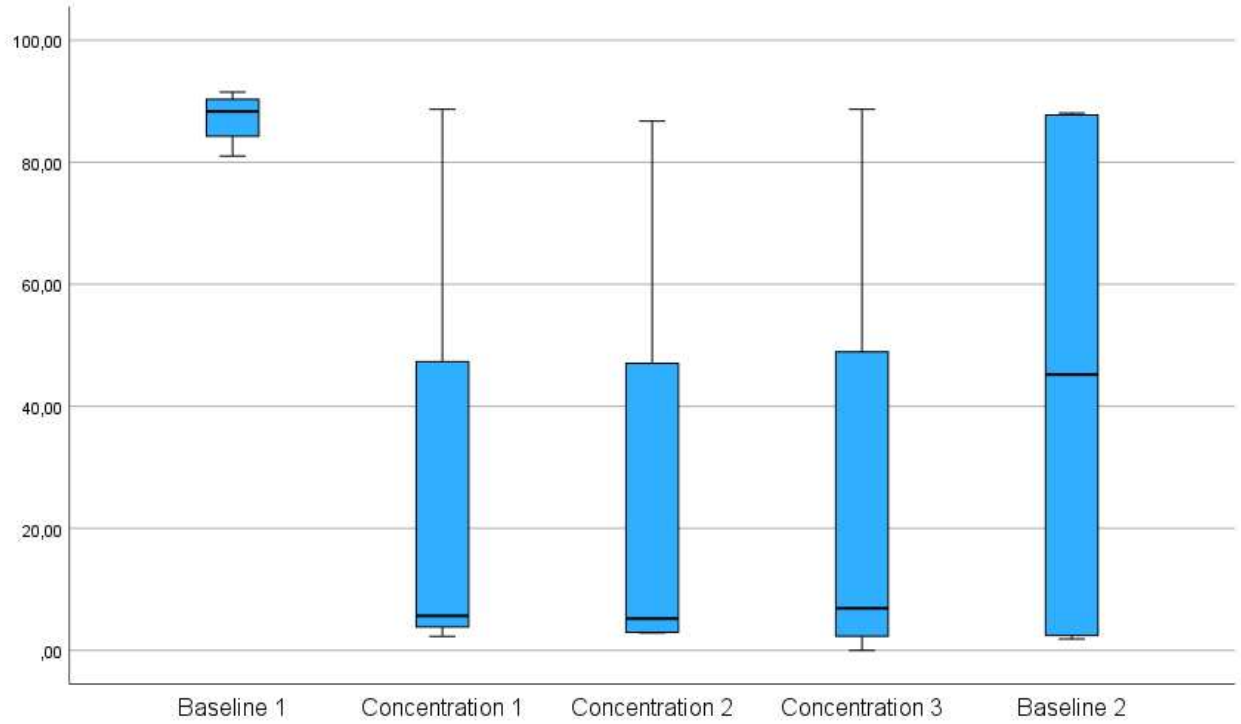

Figure 17: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 1mM arachidonic acid

Collagen-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,59	88,2	79,32	36,65	86,03
2	84,4	83,9	78,06	44,58	87,24
3	90,85	54,03	28,4	85,63	87,71
6	88,31	87,72	85,95	82,27	88,78
Q1	87,33	76,43	65,65	42,60	86,94
Median	89,58	85,81	78,69	63,43	87,48
Q3	91,04	87,84	80,98	83,11	87,98

Table 9: LTA-Results after addition of Tramadol and Metamizol in rising concentrations and collagen

In this test, collagen was used as the agonist at a concentration of 2µg/ ml. The baseline platelet function, measured without the addition of tramadol, was 89,58% (87,33% –

91,04%). When tramadol (200 ng/ml) was combined with increasing concentrations of metamizol (300 µg/ml, 600 µg/ml, and 900 µg/ml), the following median platelet function values were observed:

- **300 µg/ml metamizol:** 85,81% (76,43% – 87,84%)
- **600 µg/ml metamizol:** 78,69% (65,65% – 80,98%)
- **900 µg/ml metamizol:** 63,43% (42,60% – 83,11%)

In a second control group, which did not receive tramadol or metamizol, the median platelet function was 87,48% (86,94% – 87,98%).

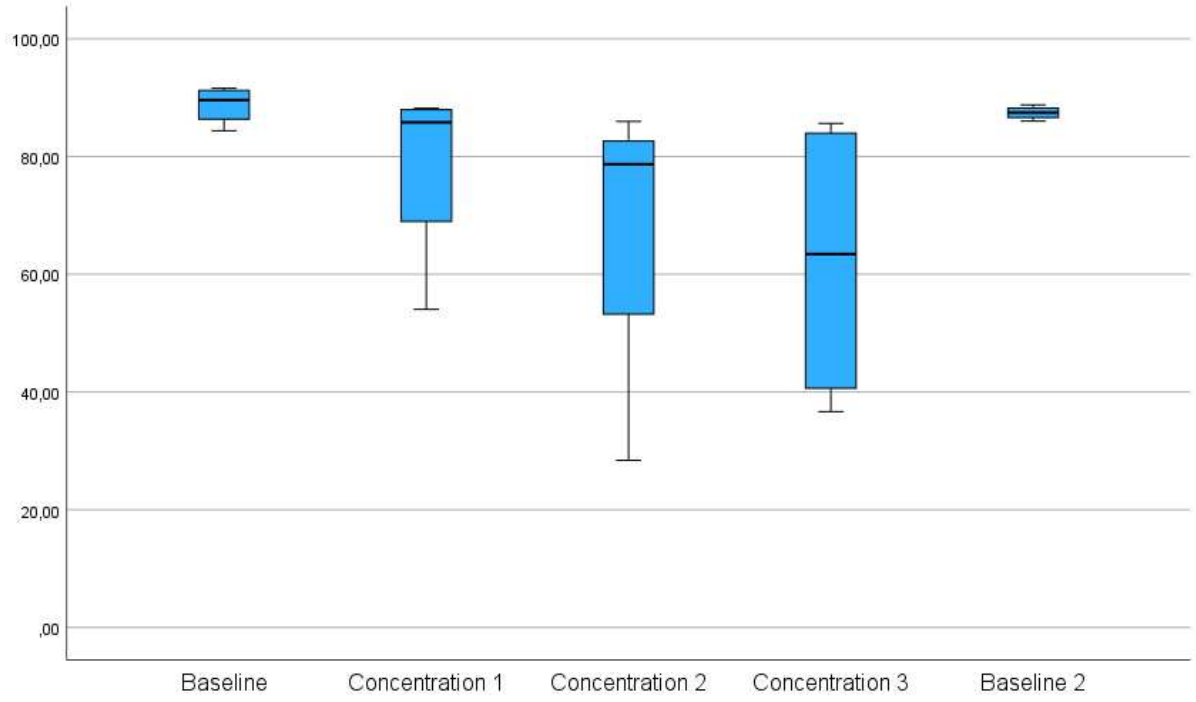

Figure 18: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with collagen 2µg/ ml

A statistically significant difference in thrombocyte function after collagen-activation and addition of tramadol in combination with metamizol was observed between the different concetration groups, p=0,016. A post-hoc analysis between the different groups was

performed and adjusted significance was always greater than 0,05, between Baseline 1 and Concetration 3 it was 0,073.

Kendall´s W was 0,763.

Ristocetin-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,45	89,16	83,65	86,82	90,45
2	83,59	84,44	83,14	84,33	89,28
3	90,77	88,54	86,28	87,21	91,04
6	87,59	87,37	85,56	84,2	86,49
Q1	86,59	86,65	83,52	84,30	88,58
Median	89,18	87,96	84,61	85,58	89,87
Q3	90,94	88,70	85,74	86,92	90,60

Table 10: LTA-results after addition of tramadol and metamizol in rising concentrations and ristocetin

In this test, ristocetin was used as the agonist at a concentration of 1,2 mg/ ml. The baseline platelet function, measured without the addition of tramadol, was 89,18% (86,59% – 90,94%). When tramadol (200 ng/ml) was combined with increasing concentrations of metamizol (300 µg/ml, 600 µg/ml, and 900 µg/ml), the following median platelet function values were observed:

- **300 µg/ml metamizol:** 87,96% (86,65% – 88,70%)
- **600 µg/ml metamizol:** 84,61% (83,52% – 85,74%)
- **900 µg/ml metamizol:** 85,58% (84,30% – 86,92%)

In a second control group, which did not receive tramadol or metamizol, the median platelet function was 89,87% (88,58% – 90.60%).

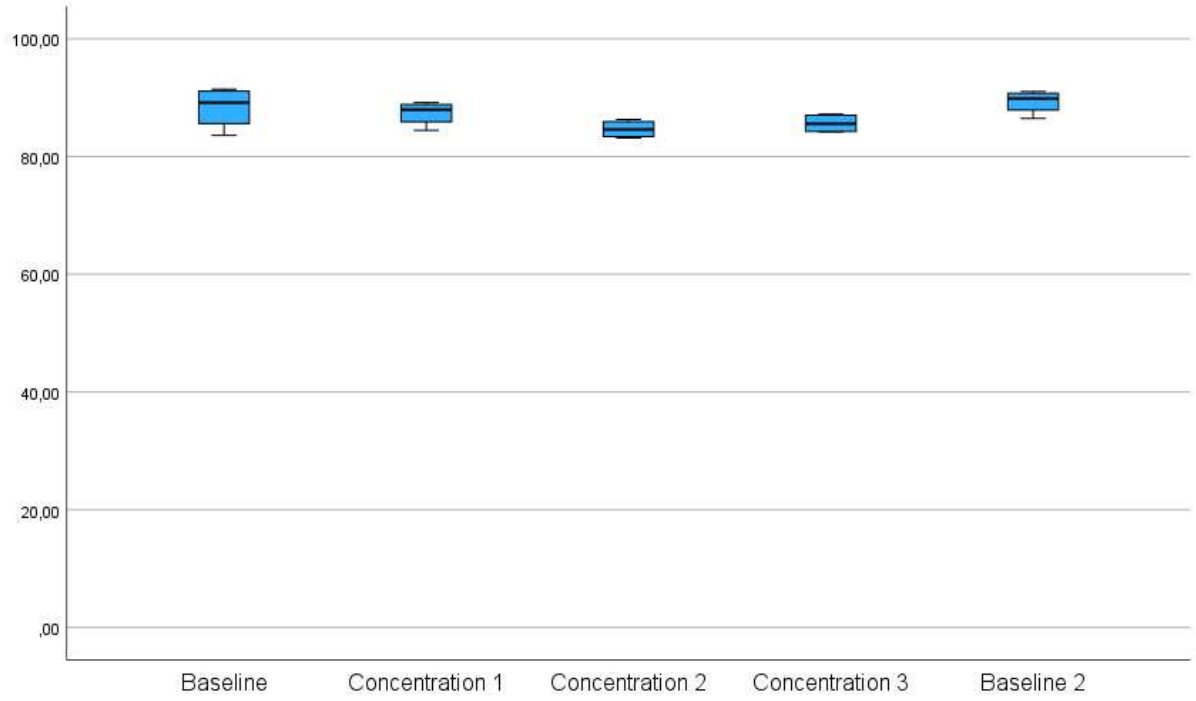

Figure 19: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with Ristocetin 1,2mg/ ml

A statistically significant difference in thrombocyte function after ristocetin-activation and addition of tramadol in combination with metamizol was observed between the different concetration groups, p=0,027. A post-hoc analysis between the different groups was performed and adjusted significance was always greater than 0,05.

Kendall´s W was 0,688.

TRAP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	89,96	90	86,99	86,06	92,1
2	90,12	89,6	84,12	86,47	88,33
3	90,06	82,72	82,67	91,34	91,4
6	88,29	85,29	88,8	89,43	88
Q1	89,54	84,65	83,76	86,37	88,25
Median	90,01	87,45	85,56	87,95	89,87
Q3	90,08	89,70	87,44	89,91	91,58

Table 11: LTA-results after addition of tramadol and metamizol in rising concentrations and TRAP

In this test, TRAP was used as the agonist at a concentration of 50µM. The baseline platelet function, measured without the addition of tramadol, was 90,01% (89,54% – 90,08%). When tramadol (200 ng/ml) was combined with increasing concentrations of metamizol (300 µg/ml, 600 µg/ml, and 900 µg/ml), the following median platelet function values were observed:

- **300 µg/ml metamizol:** 87,45% (84,65% – 89,70%)
- **600 µg/ml metamizol:** 85,56% (83,76% – 87,44%)
- **900 µg/ml metamizol:** 87,95% (86,37% – 89,91%)

In a second control group, which did not receive tramadol or metamizol, the median platelet function was 89,87% (88,25% – 91,58%).

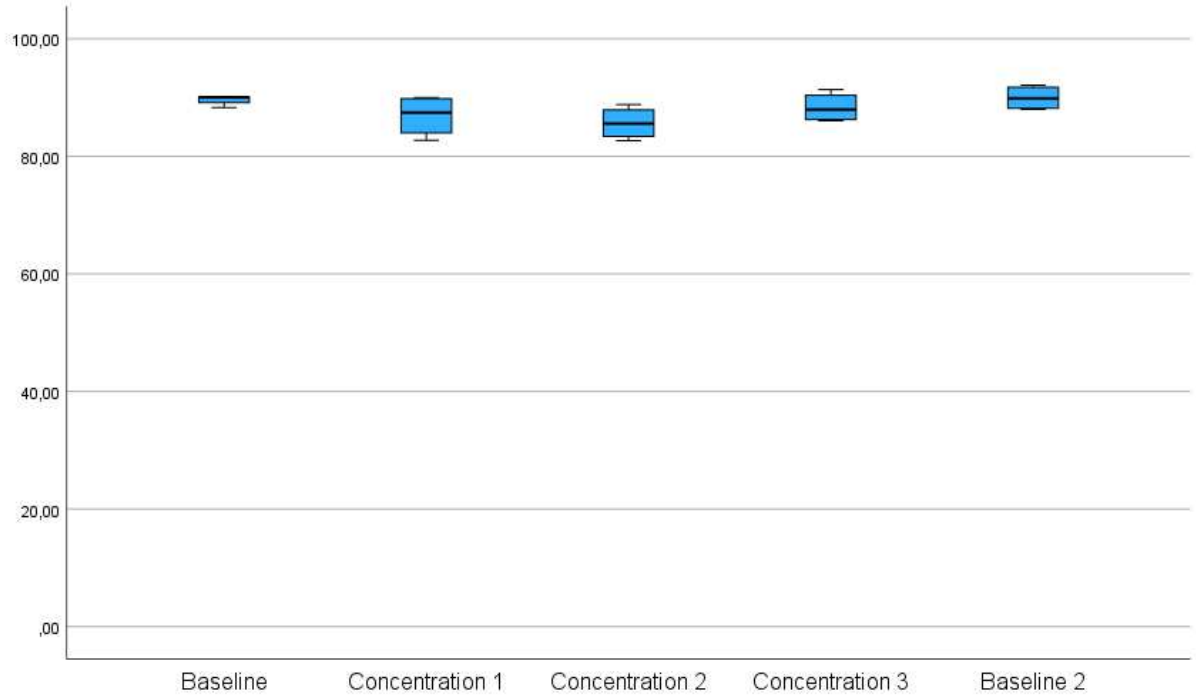

Figure 20: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation, using activation with 50µM TRAP

A statistically significant difference in thrombocyte function after activation with TRAP and addition of tramadol in combination with metamizol was not observed between the different concetration groups, p=0,558.

Kendall´s W was 0,188.

Fentanyl

ADP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	88,17				76,20
2	82,00	83,76			35,27
3	103,58	104,62	95,73	6,91	105,32
4	95,78	83,87	88,11	83,60	83,78
5	87,59	89,64	88,23	80,71	87,04
6	91,00	89,11	86,04	87,95	92,79
Q1	87,74	83,87	87,59	62,26	78,10
Median	89,59	89,11	88,17	82,16	85,41
Q3	94,59	89,64	90,11	84,69	91,35

Table 12: LTA-results after addition of tramadol and fentanyl in rising concentrations and ADP

In this test, ADP was used as an agonist at a concentration of 10 µM. The baseline platelet function, measured without the addition of tramadol, was 89,59% (87,74% – 94,59%). When tramadol (200 ng/ml) was combined with increasing concentrations of fentanyl (3000 ng/ml, 6000 ng/ml, and 9000 ng/ml), the following median platelet function values were observed:

- **3000 ng/ml fentanyl:** 89,11% (83,87% – 89,64%)
- **6000 ng/ml fentanyl:** 88,17% (87,59% – 90,11%)
- **9000 ng/ml fentanyl:** 82,16% (62,26% – 84,69%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 85,41% (78,10% – 91,35%).

A statistically significant difference in thrombocyte function after activation with ADP and addition of tramadol in combination with fentanyl was not observed between the different concetration groups, p=0,075.

Kendall´s W was 0,424.

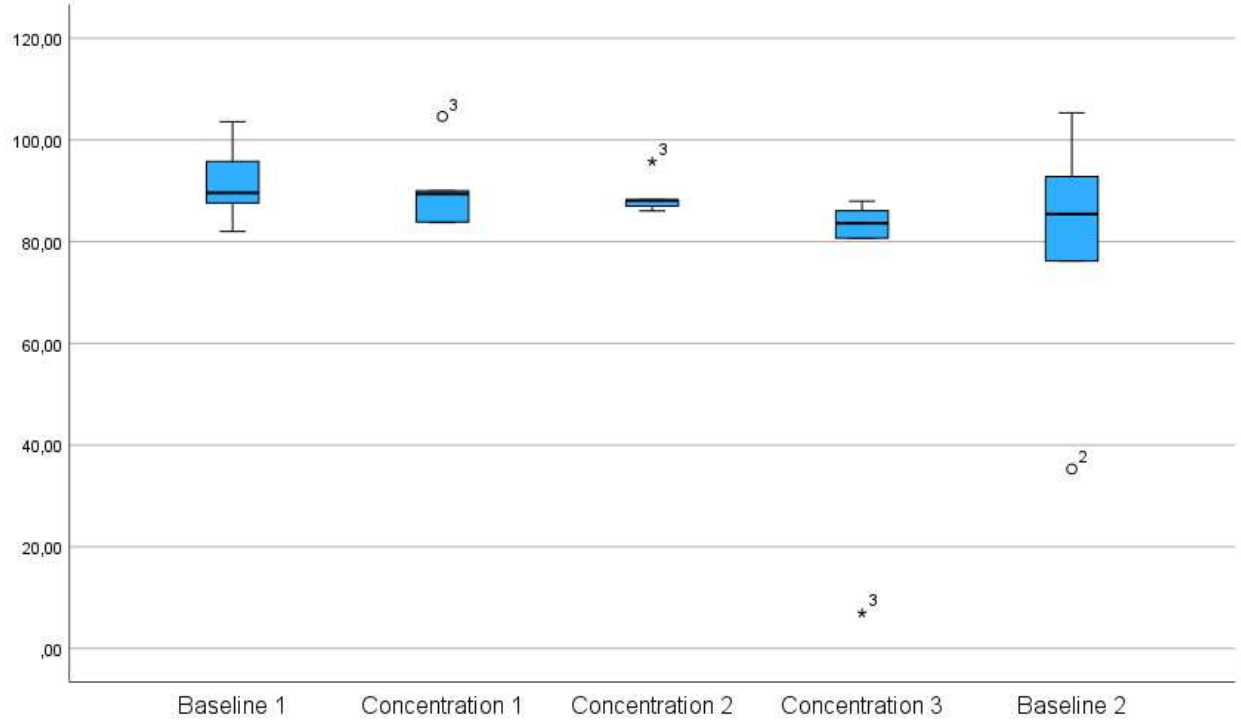

Figure 21: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 10µM ADP

Arachidonic-Acid-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,69				1,68
2	81,67		85,65		1,64
3	108,11	100,72	88,25	102,42	106,38
4	95,25	90,14	91,17	87,71	86,52
5	90,43	88,72	87,79	89,35	79,54
6	90,97	89,36	88,80	91,95	92,55
Q1	90,57	89,20	87,79	88,94	21,15
Median	91,33	89,75	88,25	90,65	83,03
Q3	94,36	92,79	88,80	94,57	91,04

Table 13: LTA-results after addition of tramadol and fentanyl in rising concentrations and arachidonic acid

In this test, arachidonic acid was used as agonist at a concentration of 1mM. The baseline platelet function, measured without the addition of tramadol, was 91,33% (90,57% – 94,36%). When tramadol (200 ng/ml) was combined with increasing concentrations of fentanyl (3000 ng/ml, 6000 ng/ml, and 9000 ng/ml), the following median platelet function values were observed:

- **3000 ng/ml fentanyl:** 89,75% (89,20% – 92,79%)
- **6000 ng/ml fentanyl:** 88,25% (87,79% – 88,80%)
- **9000 ng/ml fentanyl:** 90,65% (88,94% – 94,57%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 83,03% (21,15% – 91,04%).

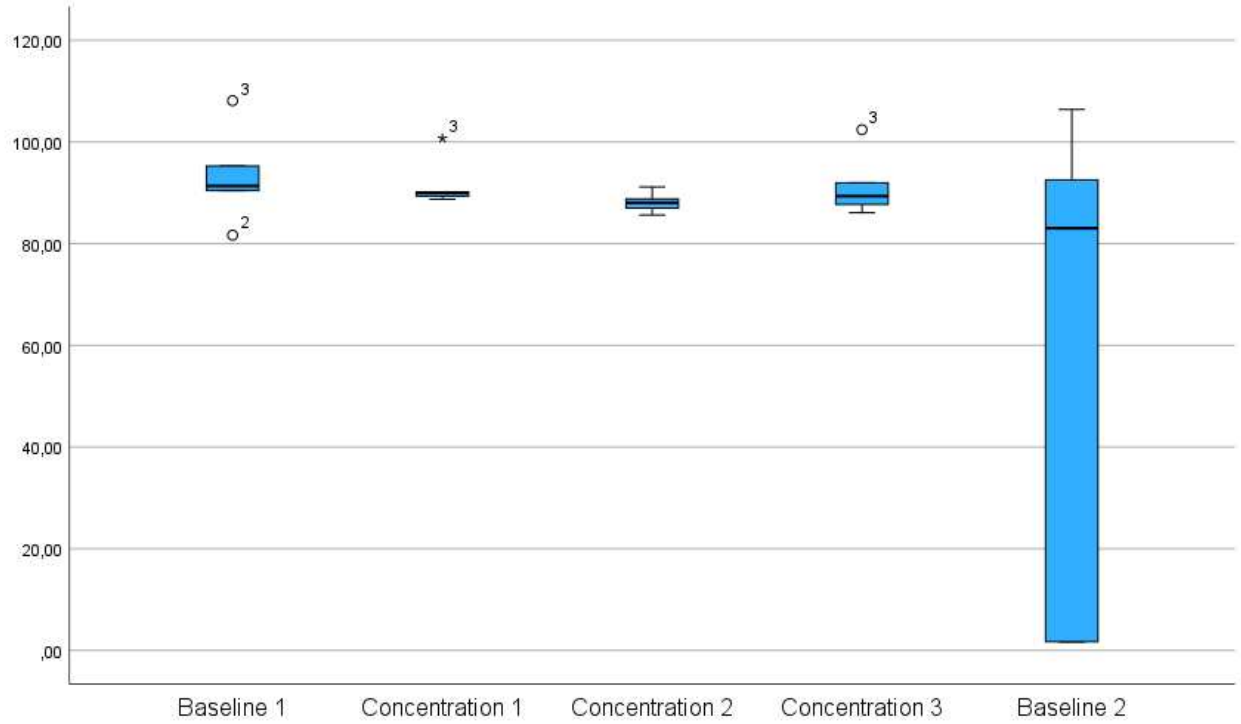

Figure 22: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 1mM arachidonic acid

A statistically significant difference in thrombocyte function after activation with arachidonic acid and addition of tramadol in combination with fentanyl was not observed between the different concetration groups, p=0,126.

Kendall´s W was 0,360.

Collagen-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	87,01				77,42
2	82,59	84,06			28,62
3	111,19	107,3	99,89	108,99	105,58
4	94,9	87,66	88,32	87,82	83,31
5	87,83	89,45	86,66	86,51	87,77
6	89,77	88,54	80,62	86,48	88,66
Q1	87,22	87,66	85,15	86,50	78,89
Median	88,80	88,54	87,49	87,17	85,54
Q3	93,62	89,45	91,21	93,11	88,44

Table 14: LTA-results after addition of tramadol and fentanyl in rising concentrations and collagen

In this test, collagen was used as the agonist at a concentration of 2µg/ ml. The baseline platelet function, measured without the addition of tramadol, was 88,80% (87,22% – 93,62%). When tramadol (200 ng/ml) was combined with increasing concentrations of fentanyl (3000 ng/ml, 6000 ng/ml, and 9000 ng/ml), the following median platelet function values were observed:

- **3000 ng/ml fentanyl:** 88,54% (87,66% – 89,45%)
- **6000 ng/ml fentanyl:** 87,49% (85,15% – 91,21%)
- **9000 ng/ml fentanyl:** 87,17% (86,50% – 93,11%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 85,54% (78,89% – 88,444%).

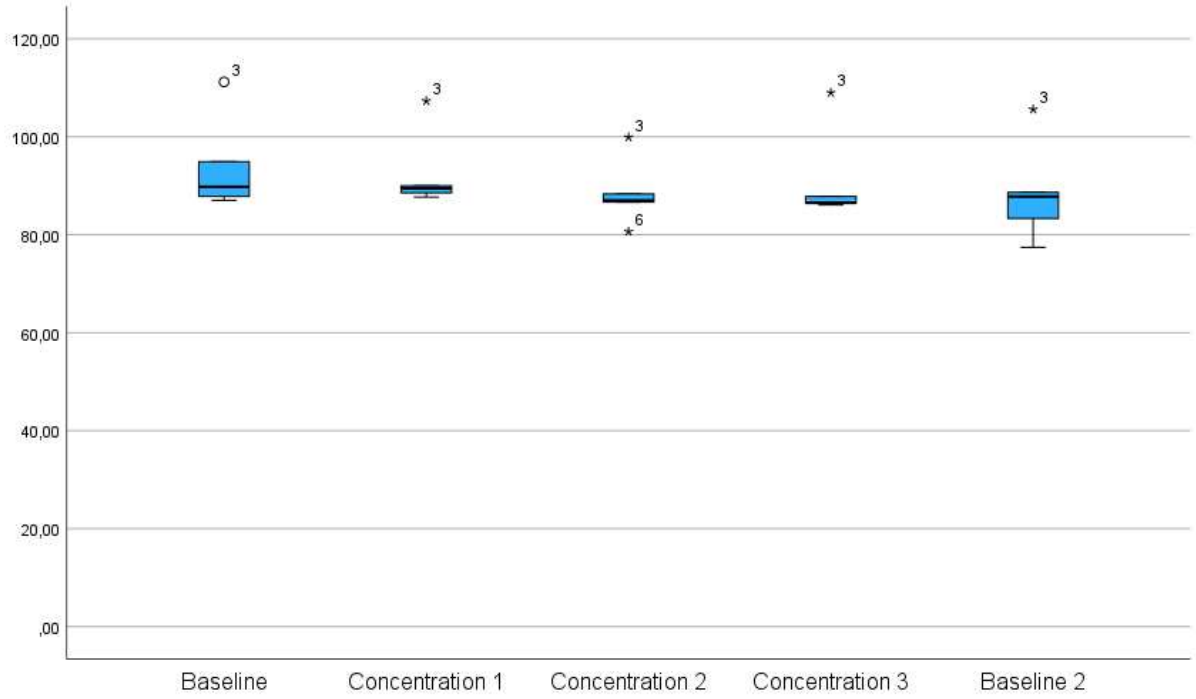

Figure 23: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with collagen 2µg/ ml

A statistically significant difference in thrombocyte function after activation with collagen and addition of tramadol in combination with fentanyl was not observed between the different concetration groups, p=0,058.

Kendall´s W was 0,456.

Ristocetin-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	90,83				70,19
2	84,58	75,86			42,84
3	109	103,88	96,59	7,71	102,76
4	92,95	85,78	92,78	91,32	86,69
5	86,37	88,32	87,09	84,93	85,14
6	87,63	89,15	85,59	88,66	90,18
Q1	86,69	85,78	86,72	65,63	73,93
Median	89,23	88,32	89,94	86,80	85,92
Q3	92,42	89,15	93,73	89,33	89,31

Table 15: LTA-results after addition of tramadol and fentanyl in rising concentrations and ristocetin

In this test, ristocetin was used as the agonist at a concentration of 1,2mg/ ml. The baseline platelet function, measured without the addition of tramadol, was 89,23% (86,69% – 92,42%). When tramadol (200 ng/ml) was combined with increasing concentrations of fentanyl (3000 ng/ml, 6000 ng/ml, and 9000 ng/ml), the following median platelet function values were observed:

- **3000 ng/ml fentanyl:** 88,32% (85,78% – 89,15%)
- **6000 ng/ml fentanyl:** 89,94% (86,72% – 93,72%)
- **9000 ng/ml fentanyl:** 86,80% (65,63% – 89,33%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 85,92% (73,93% – 89,31%).

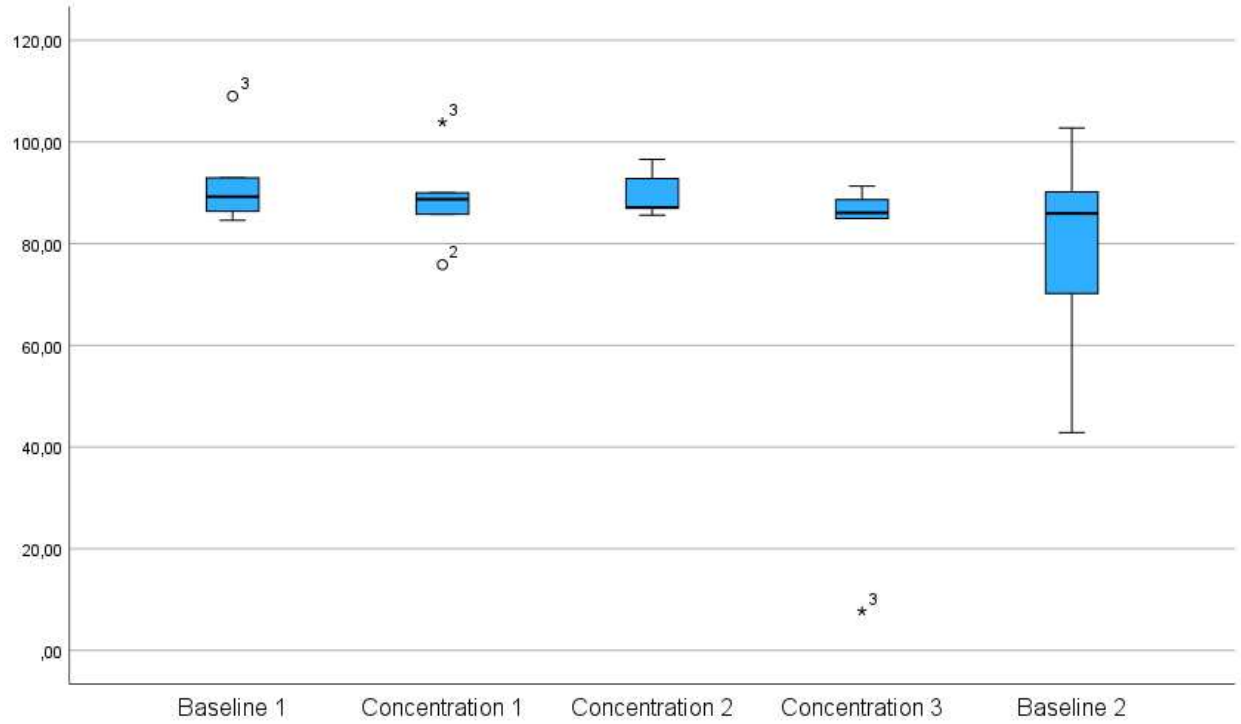

Figure 24: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with Ristocetin 1,2mg/ ml

A statistically significant difference in thrombocyte function after activation with ristocetin and addition of tramadol in combination with fentanyl was not observed between the different concetration groups, p=0,275.

Kendall´s W was 0,256.

TRAP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	89,24				82,68
2	88,25	81,58			61,46
3	108,6	104,56	99,18	102,08	105,91
4	93,68	88,11	91,4	87,04	86,28
5	89,55	91,17	88,01	90,55	89,2
6	87,28	91,32	89,98	86,82	90,04
Q1	88,50	88,11	89,49	86,99	83,58
Median	89,40	91,17	90,69	88,80	87,74
Q3	92,65	91,32	93,35	93,43	89,83

Table 16: LTA-results after addition of tramadol and fentanyl in rising concentrations and TRAP

In this test, TRAP was used as the agonist at a concentration of 50µM. The baseline platelet function, measured without the addition of tramadol, was 89,40% (88,50% – 92,65%). When tramadol (200 ng/ml) was combined with increasing concentrations of fentanyl (3000 ng/ml, 6000 ng/ml, and 9000 ng/ml), the following median platelet function values were observed:

- **3000 ng/ml fentanyl:** 91,17% (88,11% – 91,32%)
- **6000 ng/ml fentanyl:** 90,69% (89,49% – 93,35%)
- **9000 ng/ml fentanyl:** 88,80% (86,99% – 93,43%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 87,74% (83,58% – 89,83%).

A statistically significant difference in thrombocyte function after activation with ristocetin and addition of tramadol in combination with fentanyl was not observed between the different concetration groups, p=0,142.

Kendall´s W was 0,344.

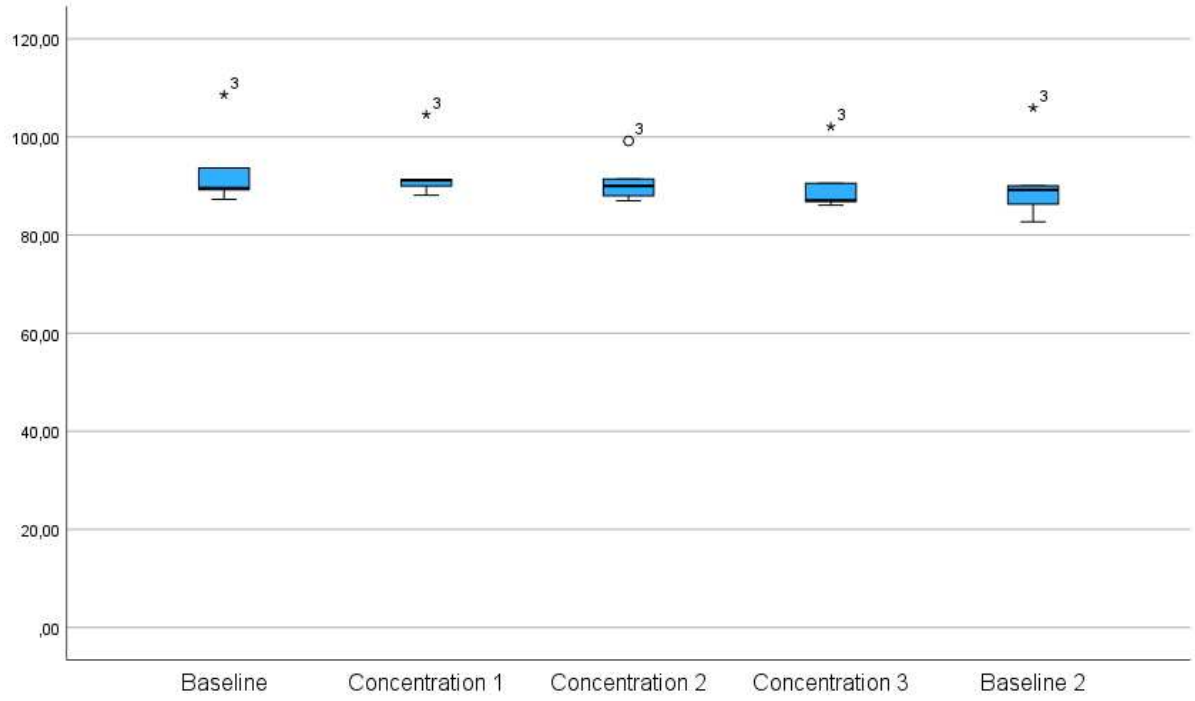

Figure 25: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation, using activation with 50µM TRAP

Ibuprofen

ADP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	85,42	80,27			85,06
2	91,95	79,92	78,10	81,65	90,07
3	88,52	86,39	83,39	81,67	90,47
4	91,23	80,00	82,78	74,35	89,58
5	84,67	68,50	67,65	71,89	86,85
6	89,85	75,80	80,84	76,40	88,21
Q1	86,20	76,83	78,10	74,35	87,19
Median	89,19	79,96	80,84	76,40	88,90
Q3	90,89	80,20	82,78	81,65	89,95

Table 17: LTA-Results after addition of Tramadol and ibuprofen in rising concentrations and ADP

In this test, ADP was used as an agonist at a concentration of 10 µM. The baseline platelet function, measured without the addition of tramadol, was 89,19% (86,20% – 90,89%). When tramadol (200 ng/ml) was combined with increasing concentrations of ibuprofen (60 µg/ml, 120 µg/ml, and 180 µg/ml), the following median platelet function values were observed:

- **60 µg/ ml ibuprofen:** 79,96% (76,83% – 80,20%)
- **120 µg/ ml ibuprofen**: 80,84% (78,10% – 83,78%)
- **180 µg/ ml ibuprofen:** 76,40% (74,35% – 81,65%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 88,90% (87,19% – 89,95%).

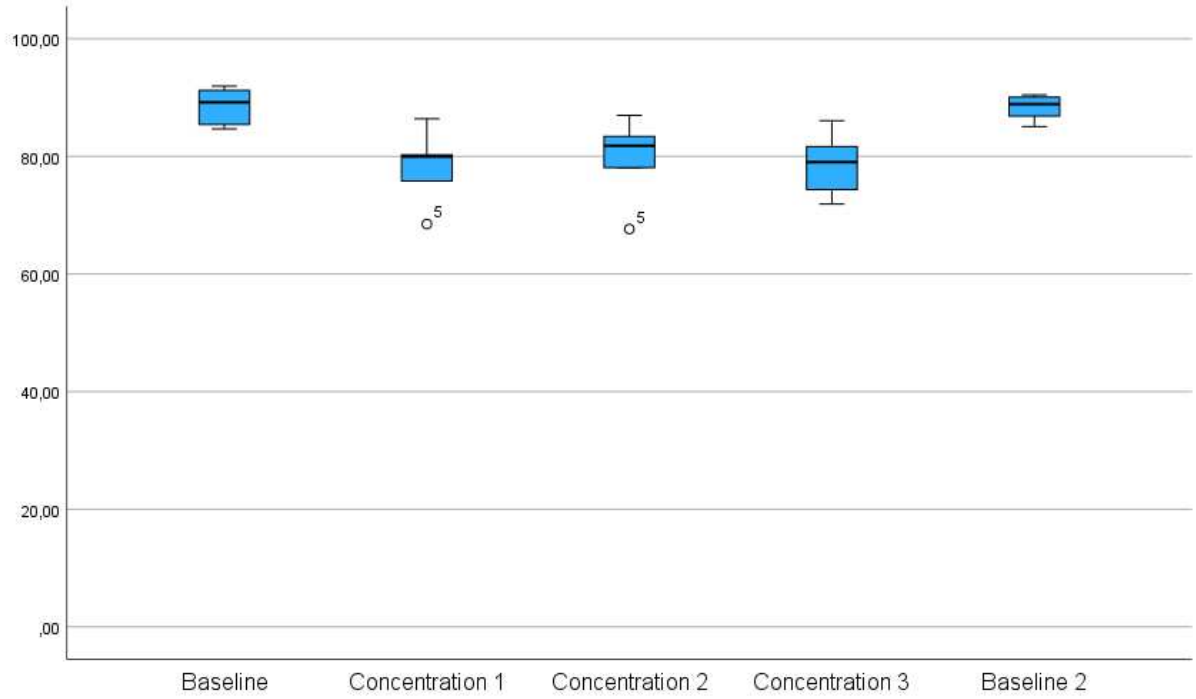

Figure 26: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 10µM ADP

A statistically significant difference in thrombocyte function after activation with ADP and addition of tramadol in combination with ibuprofen was observed between the different concetration groups, p=0,021. In a post-hoc analysis p was greater 0,05 in all analysed groups.

Kendall´s W was 0,483.

Arachidonic-Acid-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	81,19	5,88			86,14
2	92,57	8,48	4,81	7,66	91,81
3	93,08	8,15	3,75	5,04	89,60
4	91,21	2,51	5,67	0,59	90,32
5	86,22	3,03	0,17	2,53	88,57
6	90,99	0,00	3,61	1,87	89,64
Q1	87,41	2,64	3,61	1,87	88,83
Median	91,10	4,46	3,75	2,53	89,62
Q3	92,23	7,58	4,81	5,04	90,15

Table 18: LTA-Results after addition of Tramadol and ibuprofen in rising concentrations and arachidonic acid

In this test, arachidonic acid was used as agonist at a concentration of 1mM. The baseline platelet function, measured without the addition of tramadol, was 91,10% (87,41% – 92,23%). When tramadol (200 ng/ml) was combined with increasing concentrations of ibuprofen (60 µg/ml, 120 µg/ml, and 180 µg/ml), the following median platelet function values were observed:

- **60 µg/ ml ibuprofen:** 4,46% (2,64% – 7,58%)
- **120 µg/ ml ibuprofen**: 3,75% (3,61% – 4,81%)
- **180 µg/ ml ibuprofen:** 2,53% (1,87% – 5,04%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 89,62% (88,83% – 90,15%).

A statistically significant difference in thrombocyte function after activation with arachidonic acid and addition of tramadol in combination with ibuprofen was observed between the

different concetration groups, p=0,013. In a post-hoc analysis p was greater 0,05 in all analysed groups.

Kendall´s W was 0,528.

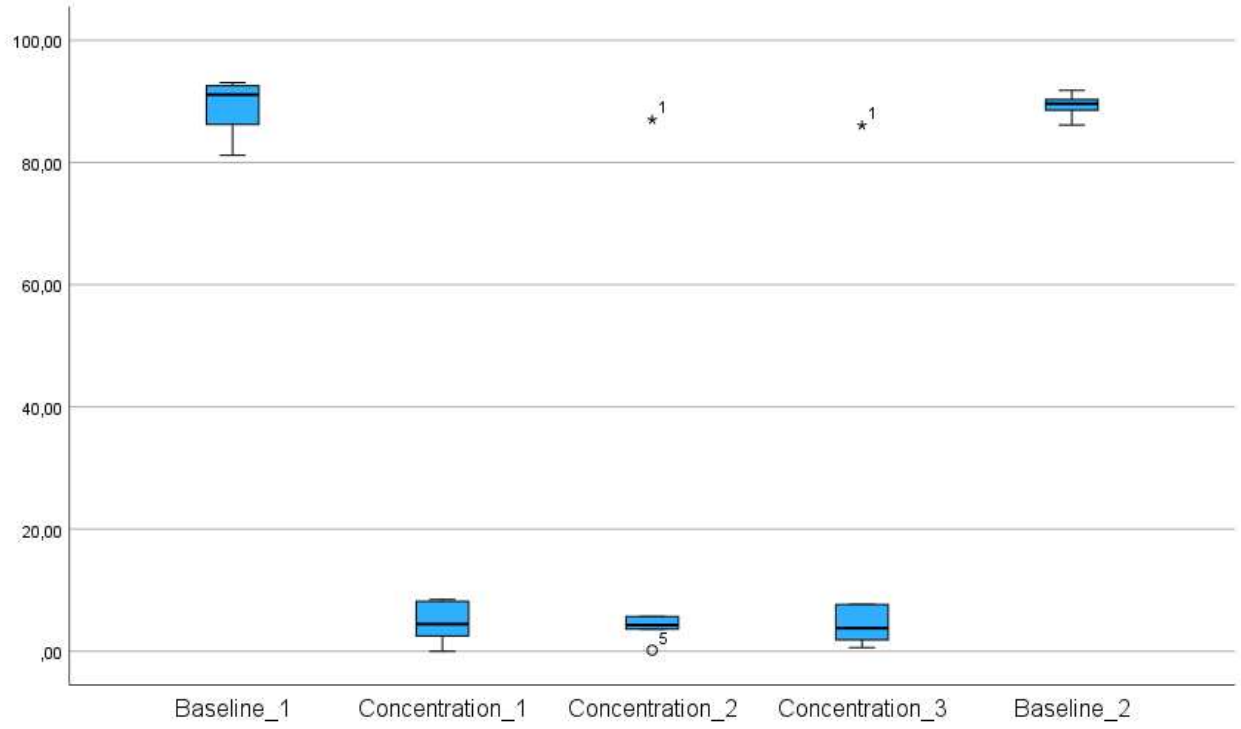

Figure 27: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with 1mM arachidonic acid

Collagen-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	84,43	80,46			83,09
2	90,66	87,25	57,93	58,46	91,05
3	88,33	91,59	75,62	64,79	89,97
4	92,55	76,76	41,13	30,11	90,37
5	86,82	76,08	61,22	60,71	88,35
6	91,05	87,65	76,98	63,01	90,31
Q1	87,20	77,69	57,93	58,46	88,76
Median	89,50	83,86	61,22	60,71	90,14
Q3	90,95	87,55	75,62	63,01	90,36

Table 19: LTA-results after addition of tramadol and ibuprofen in rising concentrations and collagen

In this test, collagen was used as the agonist at a concentration of 2µg/ ml. The baseline platelet function, measured without the addition of tramadol, was 89,50% (87,20% – 90,95%). When tramadol (200 ng/ml) was combined with increasing concentrations of ibuprofen (60 µg/ml, 120 µg/ml, and 180 µg/ml), the following median platelet function values were observed:

- **60 µg/ ml ibuprofen:** 83,86% (77,69% – 87,55%)
- **120 µg/ ml ibuprofen**: 61,22% (57,93% – 75,62%)
- **180 µg/ ml ibuprofen:** 60,71% (58,46% – 63,01%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 90,14% (88,76% – 90,36%).

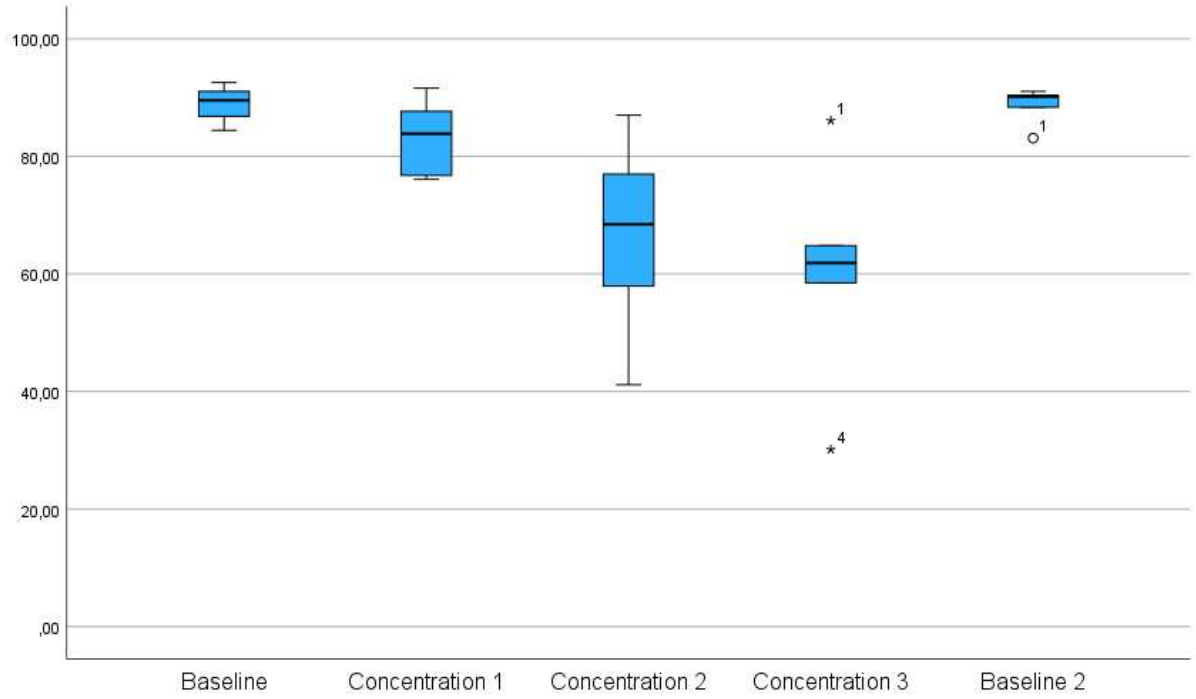

Figure 28: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with collagen 2µg/ ml

A statistically significant difference in thrombocyte function after activation with collagen and addition of tramadol in combination with ibuprofen was observed between the different concetration groups, p=0,038. In a post-hoc analysis p was greater 0,05 in all analysed groups.

Kendall´s W was 0,422.

TRAP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	85,49	82,43			84,20
2	90,48	91,79	87,75	89,48	91,94
3	92,85	93,24	86,06	88,47	87,54
4	91,35	91,34	84,52	85,17	92,64
5	87,84	84,95	82,49	79,87	86,50
6	90,92	89,70	86,24	87,31	87,68
Q1	88,50	86,14	84,52	85,17	86,76
Median	90,70	90,52	86,06	87,31	87,61
Q3	91,24	91,68	86,24	88,47	90,88

Table 20: LTA-results after addition of tramadol and ibuprofen in rising concentrations and TRAP

In this test, TRAP was used as the agonist at a concentration of 50µM. The baseline platelet function, measured without the addition of tramadol, was 90,70% (88,50% – 91,24%). When tramadol (200 ng/ml) was combined with increasing concentrations of ibuprofen (60 µg/ml, 120 µg/ml, and 180 µg/ml), the following median platelet function values were observed:

- **60 µg/ ml ibuprofen:** 90,52% (86,14% – 91,68%)
- **120 µg/ ml ibuprofen**: 86,06% (84,52% – 86,24%)
- **180 µg/ ml ibuprofen:** 87,31% (85,17% – 88,47%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 87,61% (86,76% – 90,88%).

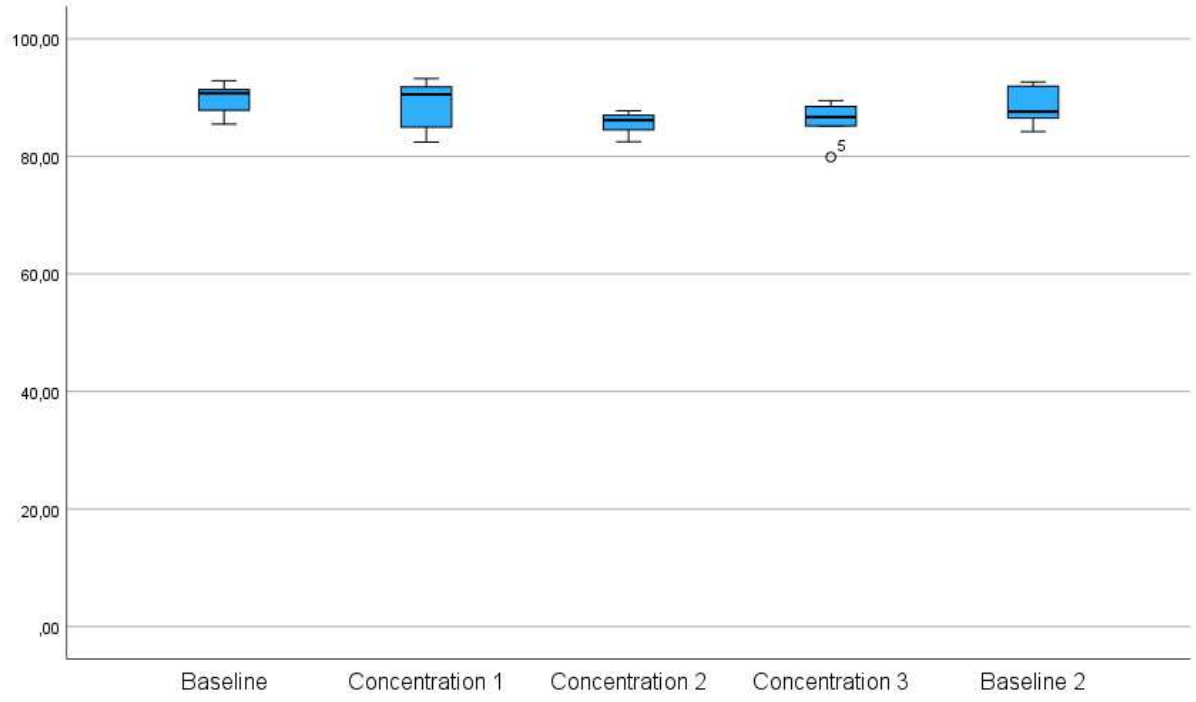

Figure 29: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation, using activation with 50µM TRAP

A statistically significant difference in thrombocyte function after activation with TRAP and addition of tramadol in combination with ibuprofen was not observed between the different concetration groups, p=0,107.

Kendall´s W was 0,317.

Ristocetin-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	84,30	68,90			85,79
2	94,67	90,88	91,18	93,51	92,80
3	88,24	88,90	60,25	83,25	91,62
4	89,48	92,88	87,39	88,15	92,33
5	89,46	85,15	81,67	85,44	90,30
6	87,74	83,28	84,84	82,64	88,19
Q1	87,87	83,75	81,67	83,25	88,72
Median	88,85	87,03	84,84	85,44	90,96
Q3	89,48	90,39	87,39	88,15	92,15

Table 21: LTA-results after addition of tramadol and ibuprofen in rising concentrations and ristocetin

In this test, ristocetin was used as the agonist at a concentration of 1,2mg/ ml. The baseline platelet function, measured without the addition of tramadol, was 88,85% (87,87% – 89,48%). When tramadol (200 ng/ml) was combined with increasing concentrations of ibuprofen (60 µg/ml, 120 µg/ml, and 180 µg/ml), the following median platelet function values were observed:

- **60 µg/ ml ibuprofen:** 87,03% (83,75% – 90,39%)
- **120 µg/ ml ibuprofen**: 84,84% (81,67% – 87,39%)
- **180 µg/ ml ibuprofen:** 85,44% (83,25% – 88,15%)

In a second control group, which did not receive tramadol or fentanyl, the median platelet function was 88,72% (88,72% – 92,15%).

A statistically significant difference in thrombocyte function after activation with ristocetin and addition of tramadol in combination with ibuprofen was not observed between the different concetration groups, p=0,171.

Kendall´s W was 0,267.

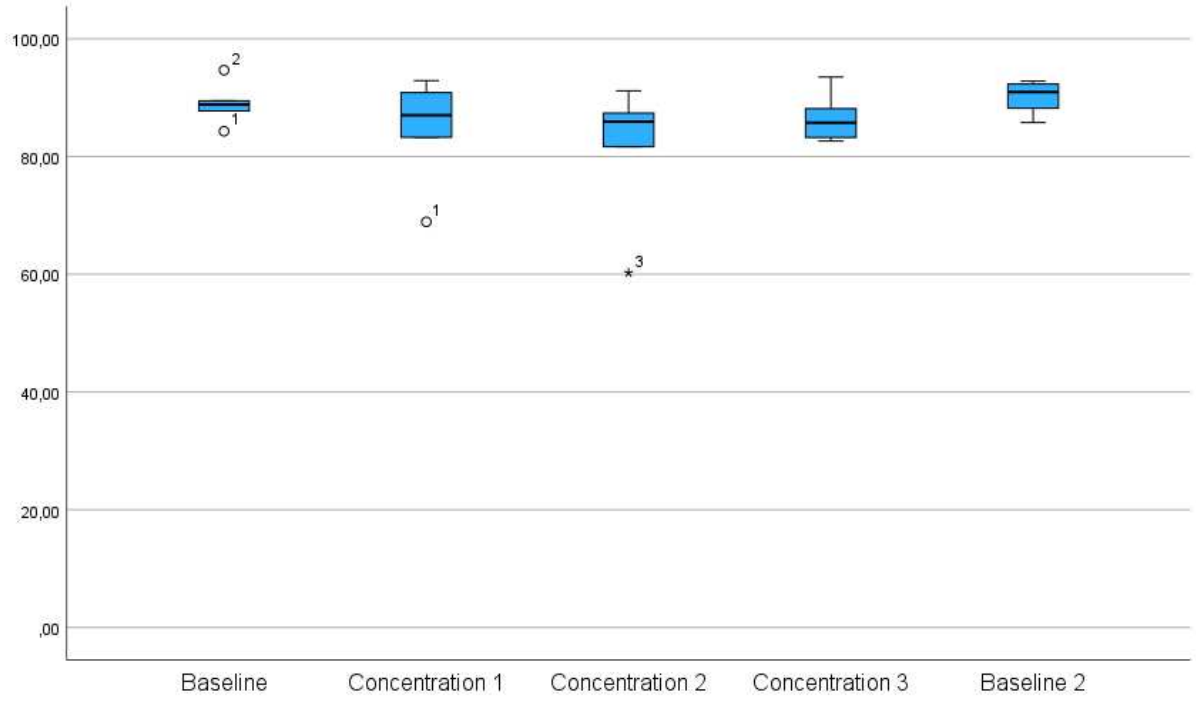

Figure 30: Results of all Dosage-groups, x-axis shows groups, y-axis shows percentage of maximal aggregation using activation with Ristocetin 1,2mg/ ml

Discussion

Answers to research questions

Research question 1

Results

The primary aim of this study was to quantify the effect of tramadol on platelet aggregation. For this purpose, the effect of tramadol in rising concentrations on platelet function was analysed in an ex-vivo setting using light-transmission aggregometry.

The null hypothesis of this study was that tramadol does not affect platelet aggregation in healthy patients.

Despite individual patients who show an impaired platelet function with certain activators, such as subjects 3 and 13 after activation with arachidonic acid, this study was not able to show a significant difference between the groups in any approach, so **the null hypothesis of our first research question could not be rejected.**

Existing literature

Given the pharmacological properties of tramadol and its influence on the neurotransmitter serotonin, a more pronounced effect was anticipated. Additionally, prior animal studies on horses by Casaella et al. as well as research on substances with comparable serotonin reuptake inhibition, such as Serotonin-Reuptake Inhibitors, have shown relevant effects. [5] However, the lack of a significant finding in this study on the first research question may be attributed to the small sample size and the ex vivo nature of the experiment, which may not fully capture the in vivo effects of tramadol on platelet function.

While these results were not statistically significant, they provide some indication that the use of tramadol could potentially have an impact on thrombocyte function in certain individuals as shown in the examples below.

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
3	87,38	5,37	7,35	4,62	3,03	89,17
13	85,86	2,22	5,82	3,55		87,29

Table 22: Cases 3 and 13 after activation with arachidonic acid as an example

Therefore this study provides indications of a noticeable inhibition of platelet function in some individuals even at the lowest tramadol concentration tested, which was within the target plasma level range of 100 - 1000 ng/ml. Additionally, an effect was observed in two individuals at the highest concentration. However, the final baseline test without tramadol addition, intended to exclude an effect due to thrombocyte damage or death, was missing for these two individuals, and they were therefore not included in the analyses. In summary, this study suggests that the use of tramadol may have an impact on thrombocyte function in certain individuals, potentially increasing the risk of bleeding, and further investigation in a larger patient cohort is warranted.

Casella et al. investigated the effect of tramadol on platelet aggregation using in vitro horse blood samples. Their findings suggest that the effect of tramadol varies between fasting and fed horses. Non-fasted horses exhibited a significant increase in platelet aggregation and enhanced clot formation upon exposure to tramadol, whereas fasting horses showed no significant changes in hemostasis compared to controls. [5]

In contrast, Bilir et al. reported the opposite in a human study. They observed a clearly slowed clot formation, as measured by thrombelastography, with increasing doses of tramadol added to blood samples in vitro. However, the clot strength in terms of maximum clot formation showed no differences between the groups. [6]

These contrasting results suggest that the effects of tramadol on platelet function may be influenced by factors such as the metabolic state of the subject or possible species-specific

differences in response to tramadol. Further research is definetely needed to better understand the nuances of tramadol's effects on haemostasis and coagulation in different patient groups such as men/women, certain pre-existing conditions such as smokers and experimental models.

Research question 2

Results

As tramadol is often administered together with other medications such as NSAIDs like ibuprofen and novalgin or other opioids such as fentanyl in the perioperative setting, an additional series of studies was performed investigating whether these combinations alter the influence of tramadol on platelet function.

In this series we could demonstrate significant effects in some settings:

- Tramadol + metamizol in rising concentrations
    - Activation with ADP: p=0,015. A post-hoc analysis between the different groups was performed and a significant difference between Baseline 1 and Concetration 2 (p=0,017) was detected.
    - Activation with collagen: p=0,016. A post-hoc analysis between the different groups was performed and adjusted significance was always greater than 0,05, between Baseline 1 and Concetration 3 it was 0,073. Kendall´s W was 0,763.
    - Activation with ristocetin: p=0,027. A post-hoc analysis between the different groups was performed and adjusted significance was always greater than 0,05. Kendall´s W was 0,688.

- Tramadol + fentanyl in rising concentrations
    - No significant effects could be demonstrated, althoug there seems tobe an effect in some patients:

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
3	103,58	104,62	95,73	6,91	105,32

Table 23: LTA-results after addition of tramadol and fentanyl in rising concentrations and ADP

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
3	109	103,88	96,59	7,71	102,76

Table 24: LTA-results after addition of tramadol and fentanyl in rising concentrations and ristocetin

- Tramadol + ibuprofen in rising concentrations
    - Activation with ADP: A statistically significant difference in thrombocyte function after activation with ADP and addition of tramadol in combination with ibuprofen was observed between the different concetration groups, p=0,021. In a post-hoc analysis p was greater 0,05 in all analysed groups. Kendall´s W was 0,483.
    - Arachidonic acid: A statistically significant difference in thrombocyte function after activation with arachidonic acid and addition of tramadol in combination with ibuprofen was observed between the different concetration groups, p=0,013. In a post-hoc analysis p was greater 0,05 in all analysed groups. Kendall´s W was 0,528.
    - Collagen: A statistically significant difference in thrombocyte function after activation with collagen and addition of tramadol in combination with ibuprofen was observed between the different concetration groups, p=0,038. In a post-hoc analysis p was greater 0,05 in all analysed groups. Kendall´s W was 0,422.

To summarise, **the null hypothesis in this research question can be partially rejected.** Depending on the addiotionally added substance, there seems to be an effect on platelet

function. Even with fentanyl, where there are no significant differences in platelet function across all groups, there does appear to be a measurable impairment of platelet function in some individuals and activators.

Effects of metamizol in existing literature

The effect of metamizol on Platelet function is well known for many years and demonstrated in an increasing nummer of studies in different settings. [51]

For example, in a study of 26 patients undergoing arthroscopic partial meniscectomy, Graff et al showed that platelet aggregation was significantly lower for 6 hours after administration of metamizole compared with patients treated with parecoxib. [52]

In a study of 96 patients with subarachnoid haemorrhage (SAH), 17 of whom received metamizole and 10 dexketoprofen, Parkhutik et al. showed that the administration of COX-inhibiting analgesics in the first few days after SAH leads to a state of hypoaggregability and influences platelet aggregation. [53]

Bozzo et al. took a very innovative approach and investigated the prohaemorrhagic potential of metamizole, ibuprofen, ketoralac and acetyl-salicylic acid (ASA) in relation to their ability to impair primary haemostasis. For this purpose, blood was incubated in vitro with each drug and perfused under different shear rates using a flow-through system. Metamizole and ketoralac were able to significantly reduce the thrombus height. [54]

In their 2014 study, Papp et al. were also able to show that metamizole reduces platelet function. Epinephrine-induced aggregation was completely inhibited in vitro at all added concentrations. An in vivo part of their study even showed that metamizole can be considered as a therapeutic alternative if, for example, ASA cannot be used in oral form and intravenously administered metamizole is an effective platelet aggregation inhibitor overall. [55]

Effects of fentanyl in existing literature

In an article on possible analgesics for the treatment of acute myocardial infarction, Chodnekar et al point out both delayed platelet inhibition by morphine and the possible influence of fentanyl, which is mentioned in this article as an alternative to morphine.[56]

Given the wide distribution of opioid receptors, it is likely that fentanyl could also affect the activity of many cells, such as platelets. Whether there are opioid receptors on thrombocytes is an open question. However, recent evidence suggests, for example, that fentanyl may interfere with the action of the P2Y12-receptor inhibitor ticagrelor. This could bear the risk of insufficient platelet inhibition. The mechanism of this phenomenon is not fully understood. [57]

In contrast, a study by Bednarek et al. found no effects on platelet stimulation when fentanyl was administered in therapeutic and supratherapeutic concentrations. The authors conclude that the delayed platelet response to oral P2Y12 inhibitors does not appear to be due to direct interactions between fentanyl and platelets. [58]

As shown in Table 22 and Table 23, there seems to be a noticeable effect in some individuals. Individual Nr 3 was a 28 yo female non-smoker, more precise definitions were not collected. Since the baseline 2 value is normal again and corresponds to the initial value, in this case the platelets must have been functional until the end of the test series. Damaged platelets can therefore be ruled out as the cause of these values. A more detailed investigation of why platelet function is inhibited by fentanyl seems necessary here, especially in view of the widespread use of fentanyl in the perioperative area. Genetic factors for this influence appear to be a possible cause.

Effects of ibuprofen in existing literature

There are also numerous studies on ibuprofen that demonstrate its antiplatelet effect. For example, De La Cruz et al. compared the pharmacodynamic in-vitro profile of dexibuprofen, ibuprofen and flurbiprofen in comparison to ASA. It was shown that all three cause platelet

inhibition in a dose-dependent manner, and the effect of dexibuprofen is even comparable to that of ASA. De La Cruz used a similar study design as in our present study, adding the active ingredients to whole blood and analysing platelet function after activation using ADP, arachidonic acid and collagen. [59]

As stated by Bozzo et al [54] In summary, the various NSAIDs tested differed in their ability to affect primary haemostasis. At the dose used, aspirin was the most potentially prohaemorrhagic NSAID, closely followed by metamizole, and ibuprofen was the NSAID with the least inhibitory effect on platelet function.

One oft he possible explanations according to the article of Bozzo et al is, that it is known that not every NSAID blocks cyclooxygenase at the same site. For this reason, the presence of active metabolites of arachidonic acid is affected differently, and consequently different platelet inhibitory effects are to be expected. [60] [61]

Although an older study, Andrioli et al found no evidence as early as 1997 that ibuprofen has an effect on the coagulation cascade, in contrast to the closely related ibuprofen flurbiprofen and diclofenac. [62]

Implications for clinical practice & future research

This study did not demonstrate a statistically significant association between increasing tramadol concentrations and impaired platelet function as assessed by ex vivo LTA platelet function tests, although there was a difference between some groups. On the other hand, in combination with other analgesics, a significant effect on platelet function was demonstrated in the test series with different activators.

As already known, metamizole and ibuprofen have an influence on platelet function, which we were also able to show with our small number of cases and also demonstrate in the discussion in comparison with numerous other studies. Whether tramadol has an enhancing effect here could not be shown. However, it seems appropriate to critically question drugs with an influence on blood coagulation, particularly in the perioperative setting and especially in operations and patients with an increased risk of haemorrhage, and to subsequently investigate the exact risks and mechanisms scientifically.

Further research with a larger cohort and a more comprehensive assessment of in vivo platelet function is warranted to better elucidate the impact of tramadol on hemostasis and its potential clinical implications, particularly in high-risk populations or in perioperative settings.

As this study stated a small effect size especially in the tramadol-only group we would suggest a larger study population, a more detailed anamnesis to identify other possible influencing factors and, as in comparable studies such as Bozzo et al [54], basic coagulation parameters such as the number of thrombocytes to ensure comparability of the individual blood samples.

Rigorous data management throughout the entire study process must also be better implemented in future studies in order to generate fewer exclusions due to missing data. In particular, 8 exclusions of 15 blood samples in the group that was only incubated with tramadol are painful and avoidable.

A more complex but certainly more accurate design for a future study would be an in-vivo study in which volunteers are administered tramadol iv. This would ensure that the platelets are not damaged and that the shortest possible time to analysis is maintained. In addition, other medications could also be tested that were not possible in our setting, such as SSRIs,

which as a prodrug must first be metabolised in vivo and converted to the active ingredient, in order to determine their influence on platelet function.

Another point that needs to be considered for future research projects: In some tests, we see a loss of platelet function due to the aging process. Since the tests were carried out during routine laboratory operations, there may have been delays. Rapid processing of the various concentrations appears to be very important for future projects.
Table 25 shows an example of a loss of function due to aging oft he thrombocytes after adition of tramadol and fentanyl in rising concentrations.

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,69				1,68
2	81,67		85,65		1,64

Table 25: LTA-results after addition of tramadol and fentanyl in rising concentrations and arachidonic acid

Strength and limitations

The strength of this study is the robust study design that is already known from other studies such as Munsterhjelm et al and a well-known and established examination method for the investigation of platelet function. At the same time, a new and innovative approach was taken with the combination of tramadol with other frequently additionally administered pain medications.

The study was limited by its small sample size, which was based on the design of similar previous studies like the studies of Martini et al or Munsterjhelm et al. Martini tried to assess the influence of ibuprofen on platelet aggregation and rotational thrombelastogram on pig blood of six pigs. [48]

Munsterjhelm et al investigated the effect of acetaminophen and parecoxib on 6 healthy male volunteers each. [1]

Additionally, as an ex vivo laboratory study, the experimental conditions may not have fully captured the in vivo effects of tramadol on platelet function. The ex vivo nature of the study could potentially lead to damage or altered effects on the thrombocytes, not representative of physiological conditions. These methodological limitations may have contributed to the lack of significant findings and warrant further investigation with a larger, more robust study design to better elucidate the impact of tramadol on platelet activity.

Conclusion

This prospective laboratory study was unable to demonstrate a statistically significant relationship between increasing tramadol concentrations and impaired platelet function, as assessed through ex vivo light transmission aggregometry testing. While the findings suggest that tramadol may not have a substantial impact on thrombocyte activity, contradicting the initial hypothesis, the small sample size and ex vivo nature of the experiment may have limited the ability to detect more subtle effects. Despite the lack of statistical significance, these results provide some indication that the use of tramadol could potentially have an impact on thrombocyte function in certain individuals, potentially increasing the risk of bleeding. Further research with a larger number of patients is urgently needed.

Appendix

A 1 – Statement of the institutional review board

Medizinische Universität Graz
Ethikkommission

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VOTUM
gültig bis 30.09.2022

EK-Nummer:	33-553 ex 20/21 1342-2021
Studientitel:	The Influence of Tramadol on platelets´ function
Prüfer:	Univ. FA Dr.med.univ. Philipp Zoidl UK f. Anästhesiologie & Intensivmed., Medizinische Universität Graz
Sponsor:	Medizinische Universität Graz, Univ. Klinik für Anästhesiologie und Intensivmedizin
Ansprechpartner:	Univ. FA Dr.med.univ. Philipp Zoidl, 8010 Graz, Auenbruggerplatz 29
CRO:	-
Antragsteller:	UK f. Anästhesiologie & Intensivmed., Medizinische Universität Graz
Ansprechpartner:	Univ. FA Dr.med.univ. Philipp Zoidl, 8036 Graz, Auenbruggerplatz 29

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 17.06.2021 behandelt. Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: keine

Zur Beurteilung vorliegende Dokumente:

Dokumente eingegangen am 14.06.2021, begutachtet im 'expedited Review' am 17.06.2021	
✓ Cover Letter Anschreiben_EK 1	14.06.2021
✓ Antragsformular ECS	14.06.2021
Originalprotokoll Studienprotokoll_Tramadol_Vers2 2	01.06.2021
Informed Consent Form PatientInneninformation_Tramadol1.0 1	26.05.2021
✓ Sonstiges: Antrag_EK_Erlass 1	14.06.2021
Dokumente eingegangen am 15.06.2021, begutachtet im 'expedited Review' am 17.06.2021	
✓ Antragsformular ECS unterschrieben	15.06.2021
Dokumente eingegangen am 07.07.2021, begutachtet im 'expedited Review' am 14.07.2021	
✓ Informed Consent Form 1.1	21.06.2021
✓ Sonstiges: Stellungnahme zur Bearbeitungsmitteilung undatiert	

✓ Letter of Authorization	24.06.2021

Dokumente eingegangen am 20.09.2021, begutachtet im 'expedited Review' am 30.09.2021

✓ Originalprotokoll 2.1	20.09.2021

Die Ethikkommission geht - rechtlich unverbindlich - davon aus, dass es sich um keine klinische Prüfung nach AMG bzw. MPG handelt.

Es handelt sich um eine Studie im Rahmen einer Dissertation.

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

zusätzliche Auflagen: Die behördlich vorgeschriebenen Maßnahmen hinsichtlich der COVID-19 Pandemie müssen beachtet werden. Der Prüfer und der Sponsor müssen in ihrem jeweiligen Wirkungskreis unter allfälliger Beachtung von Leitlinien gewährleisten, dass keine zur Bekämpfung der Pandemie benötigten Ressourcen gebunden werden bzw. ausreichend Personal vorhanden ist und die TeilnehmerInnen durch ihre Studienteilnahme keiner zusätzlichen Infektionsgefahr ausgesetzt werden.

Dieses Votum gilt für ein Jahr ab dem Datum der Ausstellung. Bei längerer Studiendauer ist rechtzeitig vor Ablauf der Gültigkeit des Votums ein Zwischenbericht vorzulegen (Berichtsformular), um eine etwaige Verlängerung zu erlangen.

Graz, 30. September 2021

Univ.Prof.DI Dr.Josef Haas
Vorsitzender

Univ.Prof.Dr.Hans Peter Dimai
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

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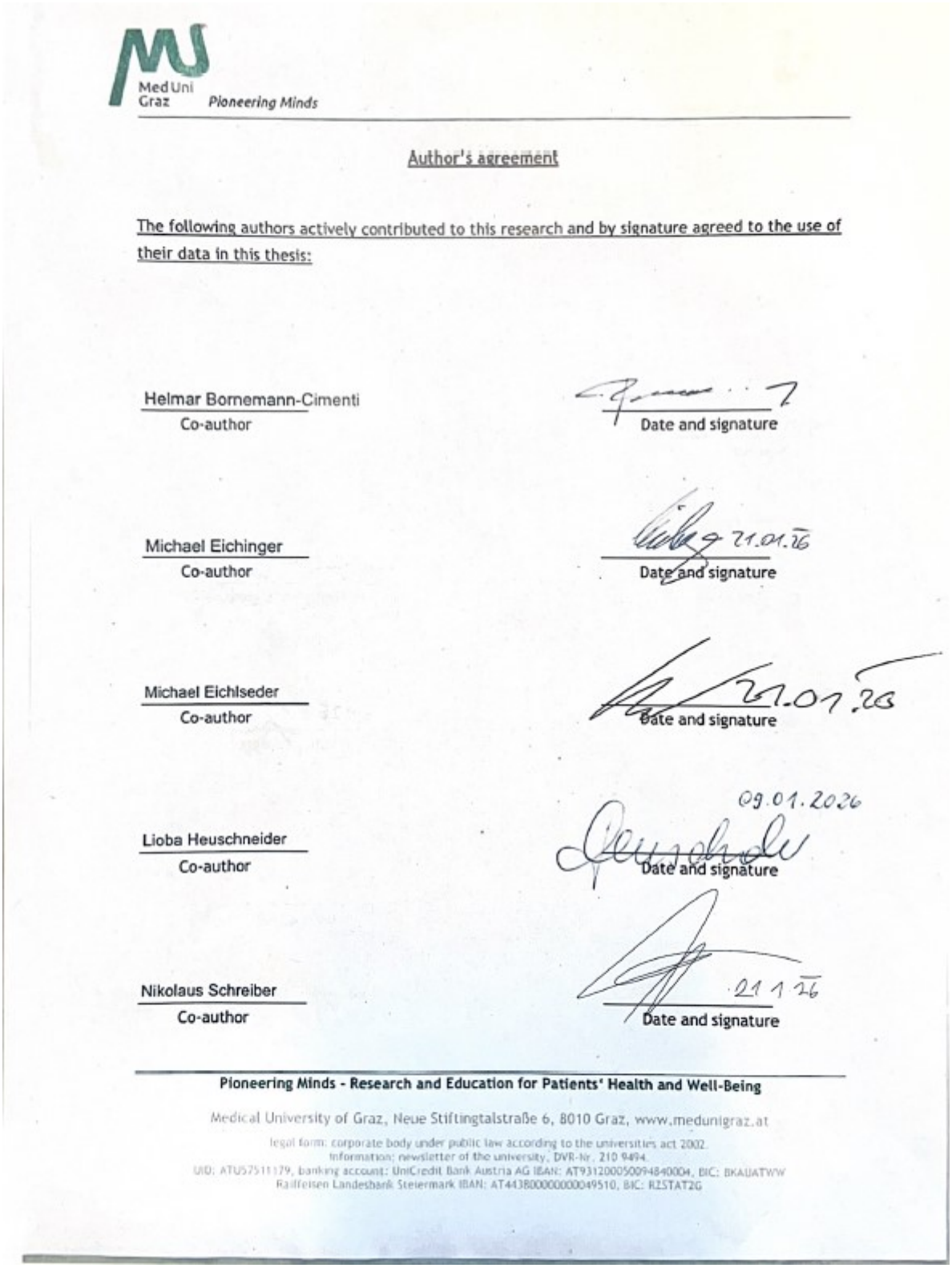
A 2 – Author´s Agreement



Med Uni Graz *Pioneering Minds*

Author's agreement

The following authors actively contributed to this research and by signature agreed to the use of their data in this thesis:

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A 3 – Complete Dataset

Tramadol

Tramadol, ADP-activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
1	86,83		64,82	64,49		
2	77,96	73,20	63,24	67,91		
3	80,49	51,03	44,07	45,36	85,81	88,95
4	94,04	84,69	91,38			93,60
5	86,62	81,87	75,53	65,31	12,28	
6	92,06	61,58			88,16	
7	83,72	81,00		79,60		77,55
8	87,20	87,63		90,28		87,05
9	93,36	90,52	88,44	89,69	92,04	87,59
10	93,22	90,05	88,93	88,47	82,41	92,67
11	94,04					
12	93,60	84,69	91,38	83,58	12,28	
13	86,62	81,87	75,53	65,31		88,16
Q1	86,62	77,10	64,82	65,31	29,81	87,32
Median	87,20	81,87	75,53	73,76	84,11	88,16
Q3	93,36	86,16	88,93	87,25	87,57	90,81

Tramadol, ARA-activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
1	1,86		9,16	3,05		
2	81,01	2,29	2,86	0,00		
3	87,38	5,37	7,35	4,62	3,03	89,17
4	94,49	1,49	83,87			96,00
5	85,86	2,22	5,82	3,55	2,37	

6	91,50	5,94			87,29	
7	2,51	1,44		2,23		3,41
8	88,35	89,81		92,15		88,15
9	88,93	92,70	89,52	88,28	7,95	87,92
10	94,81	95,00	88,70	90,32	84,65	88,92
11	94,49					
12	96,00	1,49	83,87	1,76	2,37	
13	85,86	2,22	5,82	3,55		87,29
Q1	85,86	1,86	5,82	2,44	2,54	87,61
Median	88,35	2,29	9,16	3,55	5,49	88,15
Q3	94,49	47,88	83,87	67,37	65,48	89,05

Tramadol, Collagen-activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
1	86,03		36,65	79,32		
2	84,40	83,90	78,06	44,58		
3	87,24	90,85	54,03	28,40	85,63	87,71
4	93,73	89,40	93,71			92,87
5	89,82	89,82	75,87	63,04	11,11	
6	91,59	88,20			88,24	
7	88,57	89,26		88,46		87,44
8	88,55	86,80		89,70		86,10
9	83,90	93,06	87,87	88,43	89,57	84,59
10	93,80	93,64	91,19	91,59	88,10	90,50
11	93,73					
12	92,87	89,40	93,71	86,43	11,11	
13	89,82	88,85	75,87	63,04		88,24
Q1	87,24	88,53	75,87	63,04	29,74	86,77
Median	89,82	89,40	78,06	82,88	86,87	87,71
Q3	92,87	90,34	91,19	88,45	88,21	89,37

Tramadol, TRAP-activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
1	92,10		86,06	86,99		
2	90,12	89,60	84,12	86,47		
3	88,33	90,06	82,72	82,67	91,34	91,40
4	88,42	92,94	91,04			89,05
5	87,99	88,52	89,52	87,21	20,79	
6	89,96	90,00			87,31	
7	89,95	90,26		90,52		88,92
8	88,93	89,45		89,60		86,89
9	84,16	88,19	88,75	88,46	90,36	92,63
10	89,83	94,60	89,22	93,78	89,44	89,69
11	88,42					
12	89,05	92,94	91,04	91,96	20,79	
13	87,99	88,52	89,52	87,21		87,31
Q1	88,33	88,99	86,06	87,05	37,42	88,12
Median	88,93	90,00	89,22	87,84	88,38	89,05
Q3	89,95	91,60	89,52	90,29	90,13	90,55

Tramadol, Ristocetin-activation

Pat	Baseline	Con 1	Con 2	Con 3	Con 4	Baseline 2
1	83,65		86,82	90,45		
2	83,59	84,44	83,14	84,33		
3	91,04	90,77	88,54	86,28	87,21	89,28
4	94,21	55,24	88,43			92,71
5	87,25	83,08	78,09	67,96	14,29	
6	91,45	89,16			85,52	
7	81,63	88,23		88,82		85,05
8	88,13	89,63		88,68		91,52

9	91,26	83,24	90,96	90,86	89,29	92,86
10	89,58	95,90	89,87	91,09	88,73	90,47
11	94,21					
12	92,71	55,24	88,43	49,02	14,29	
13	87,25	83,08	78,09	67,96		85,52
Q1	87,25	83,08	83,14	72,05	32,10	87,40
Median	89,58	84,44	88,43	87,48	86,37	90,47
Q3	91,45	89,40	88,54	90,04	88,35	92,12

Tramadol + Metamizol

Tramadol + Metamizol, ADP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	92,06	61,58	64,49	64,82	86,83
2	77,96	73,20	63,24	67,91	80,49
3	88,95	51,03	44,07	45,36	85,81
4	92,39				
5	89,32	87,10	83,08	85,20	86,17
6	82,86	86,02	88,29	84,38	
Q1	84,38	61,58	63,24	64,82	84,48
Median	89,14	73,20	64,49	67,91	85,99
Q3	91,38	86,02	83,08	84,38	86,34

Tramadol + Metamizol, ARA-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,50	5,94	3,05	9,16	1,86
2	81,01	2,29	2,86	0,00	87,38
3	89,17	5,37	7,35	4,62	3,03
4	90,95				

5	87,49	88,70	86,76	88,70	88,09
6	86,89	87,80	89,95	90,44	
Q1	87,04	5,37	3,05	4,62	2,74
Median	88,33	5,94	7,35	9,16	45,21
Q3	90,51	87,80	86,76	88,70	87,56

Tramadol + Metamizol, Collagen-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,59	88,20	79,32	36,65	86,03
2	84,40	83,90	78,06	44,58	87,24
3	90,85	54,03	28,40	85,63	87,71
4	89,85				
5	88,31	87,72	85,95	82,27	88,78
6	86,31	86,03	86,36	87,20	
Q1	86,81	83,90	78,06	44,58	86,94
Median	89,08	86,03	79,32	82,27	87,48
Q3	90,60	87,72	85,95	85,63	87,98

Tramadol + Metamizol, TRAP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	89,96	90,00	86,99	86,06	92,10
2	90,12	89,60	84,12	86,47	88,33
3	90,06	82,72	82,67	91,34	91,40
4	90,74				
5	88,29	85,29	88,80	89,43	88,00
6	86,82	86,60	85,50	88,73	
Q1	88,71	85,29	84,12	86,47	88,25
Median	90,01	86,60	85,50	88,73	89,87

Q3	90,11	89,60	86,99	89,43	91,58

Tramadol + Metamizol, Ristocetin-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,45	89,16	83,65	86,82	90,45
2	83,59	84,44	83,14	84,33	89,28
3	90,77	88,54	86,28	87,21	91,04
4	92,59				
5	87,59	87,37	85,56	84,20	86,49
6	86,11	84,11	84,61	86,65	
Q1	86,48	84,44	83,65	84,33	88,58
Median	89,18	87,37	84,61	86,65	89,87
Q3	91,28	88,54	85,56	86,82	90,60

Tramadol + Fentanyl

Tramadol + Fentanyl, ADP-

Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	88,17				76,20
2	82,00	83,76			35,27
3	103,58	104,62	95,73	6,91	105,32
4	95,78	83,87	88,11	83,60	83,78
5	87,59	89,64	88,23	80,71	87,04
6	91,00	89,11	86,04	87,95	92,79
Q1	87,74	83,87	87,59	62,26	78,10
Median	89,59	89,11	88,17	82,16	85,41
Q3	94,59	89,64	90,11	84,69	91,35

Tramadol + Fentanyl, ARA-

Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	91,69				1,68
2	81,67		85,65		1,64
3	108,11	100,72	88,25	102,42	106,38
4	95,25	90,14	91,17	87,71	86,52
5	90,43	88,72	87,79	89,35	79,54
6	90,97	89,36	88,80	91,95	92,55
Q1	90,57	89,20	87,79	88,94	21,15
Median	91,33	89,75	88,25	90,65	83,03
Q3	94,36	92,79	88,80	94,57	91,04

Tramadol + Fentanyl, Collagen-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	87,01				77,42
2	82,59	84,06			28,62
3	111,19	107,30	99,89	108,99	105,58
4	94,90	87,66	88,32	87,82	83,31
5	87,83	89,45	86,66	86,51	87,77
6	89,77	88,54	80,62	86,48	88,66
Q1	87,22	87,66	85,15	86,50	78,89
Median	88,80	88,54	87,49	87,17	85,54
Q3	93,62	89,45	91,21	93,11	88,44

Tramadol + Fentanyl, TRAP-

Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2

1	89,24				82,68
2	88,25	81,58			61,46
3	108,60	104,56	99,18	102,08	105,91
4	93,68	88,11	91,40	87,04	86,28
5	89,55	91,17	88,01	90,55	89,20
6	87,28	91,32	89,98	86,82	90,04
Q1	88,50	88,11	89,49	86,99	83,58
Median	89,40	91,17	90,69	88,80	87,74
Q3	92,65	91,32	93,35	93,43	89,83

Tramadol + Fentanyl, Ristocetin-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	90,83				70,19
2	84,58	75,86			42,84
3	109,00	103,88	96,59	7,71	102,76
4	92,95	85,78	92,78	91,32	86,69
5	86,37	88,32	87,09	84,93	85,14
6	87,63	89,15	85,59	88,66	90,18
Q1	86,69	85,78	86,72	65,63	73,93
Median	89,23	88,32	89,94	86,80	85,92
Q3	92,42	89,15	93,73	89,33	89,31

Tramadol + Ibuprofen

Tramadol + Ibuprofen, ADP-

Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	85,42	80,27			85,06
2	91,95	79,92	78,10	81,65	90,07

3	88,52	86,39	83,39	81,67	90,47
4	91,23	80,00	82,78	74,35	89,58
5	84,67	68,50	67,65	71,89	86,85
6	89,85	75,80	80,84	76,40	88,21
Q1	85,23	76,83	72,88	74,35	85,06
Median	89,19	79,96	80,84	76,40	87,53
Q3	91,26	80,20	83,09	81,65	89,70

Tramadol + Ibuprofen, ARA-

Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	81,19	5,88			86,14
2	92,57	8,48	4,81	7,66	91,81
3	93,08	8,15	3,75	5,04	89,60
4	91,21	2,51	5,67	0,59	90,32
5	86,22	3,03	0,17	2,53	88,57
6	90,99	0,00	3,61	1,87	89,64
Q1	87,41	2,64	3,61	1,87	88,83
Median	91,10	4,46	3,75	2,53	89,62
Q3	92,23	7,58	4,81	5,04	90,15

Tramadol + Ibuprofen, Collagen-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	84,43	80,46			83,09
2	90,66	87,25	57,93	58,46	91,05
3	88,33	91,59	75,62	64,79	89,97
4	92,55	76,76	41,13	30,11	90,37
5	86,82	76,08	61,22	60,71	88,35
6	91,05	87,65	76,98	63,01	90,31

Q1	87,20	77,69	57,93	58,46	88,76
Median	89,50	83,86	61,22	60,71	90,14
Q3	90,95	87,55	75,62	63,01	90,36

Tramadol + Ibuprofen, TRAP-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	85,49	82,43			84,20
2	90,48	91,79	87,75	89,48	91,94
3	92,85	93,24	86,06	88,47	87,54
4	91,35	91,34	84,52	85,17	92,64
5	87,84	84,95	82,49	79,87	86,50
6	90,92	89,70	86,24	87,31	87,68
Q1	88,50	86,14	84,52	85,17	86,76
Median	90,70	90,52	86,06	87,31	87,61
Q3	91,24	91,68	86,24	88,47	90,88

Tramadol + Ibuprofen, Ristocetin-Activation

Pat	Baseline	Con 1	Con 2	Con 3	Baseline 2
1	84,30	68,90			85,79
2	94,67	90,88	91,18	93,51	92,80
3	88,24	88,90	60,25	83,25	91,62
4	89,48	92,88	87,39	88,15	92,33
5	89,46	85,15	81,67	85,44	90,30
6	87,74	83,28	84,84	82,64	88,19
Q1	87,87	83,75	81,67	83,25	88,72
Median	88,85	87,03	84,84	85,44	90,96
Q3	89,48	90,39	87,39	88,15	92,15

Table 26: Complete Dataset

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