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Inhibition of platelet function after ocular administration of non-steroidal anti-inflammatory drugs

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ABSTRACT

Introduction: The use of topical NSAIDs is frequent in ophthalmology to reduce the local inflammatory reaction resulting from surgical procedures. Ocular use of some drugs was previously found to lead to significant systemic absorption with possible systemic effects. NSAIDs may enhance the hemorrhagic risk of anticoagulant and antiplatelet drugs.

Aim of our study was to evaluate the systemic effects of two NSAIDs given by eyedrops on platelet COX-1 and on ex vivo and in vivo platelet activation.

Materials and methods: 20 patients planned to undergo cataract surgery were randomized to the use of an ophthalmic solution containing Diclofenac or Indomethacin. Blood was taken at enrollment (baseline) and after 3 days of therapy (1 drop, 4 times a day).

Arachidonic Acid (AA)-induced light transmission aggregometry (LTA), PFA-100® C-EPI, circulating platelet P-Selectin expression by flow cytometry and serum and AA-induced TxB_2 production were evaluated before and after eyedrop therapy.

Results: AA (0.1–0.2 mM)-induced LTA was significantly reduced after ocular indomethacin but not after diclofenac. PFA-100® C-EPI closure time was also significantly prolonged in the indomethacin group but not in the diclofenac group. Circulating platelet P-selectin expression was significantly reduced after treatment with indomethacin compared with diclofenac. Finally, treatment with eyedrop indomethacin, but not with diclofenac, strikingly suppressed AA-induced TxB_2 generation, while treatment with diclofenac did not modify it.

Conclusions: Our data show that indomethacin administered by ophthalmic eye drops has a relevant systemic antiplatelet effect. This should be taken into account in patients under concurrent therapy with antiplatelet or anticoagulant agents.

1. Introduction

Around 95 million people worldwide are affected by cataract, and the current standard management of a visually significant cataract is surgical removal of the lens and its replacement with an intraocular artificial lens [1]. Cataract surgery is one of the most commonly performed surgeries in the world, and improvements in post-operative outcomes may carry significant public health benefit. Although advances in cataract surgery have resulted in improved outcomes, some complications, such as post-operative macular edema (PME), still occur and sometimes even lead to poor visual outcome [2,3]. Topical non-steroidal anti-inflammatory drugs (NSAIDs) are commonly employed prophylactically in patients undergoing cataract surgery to maintain intraoperative mydriasis and for the management of ocular pain and for the prevention of PME [1]. Indeed, several studies, including a recent systematic review and meta-analysis, have shown a protective effect of topical NSAIDs from cataract surgery complications [4–6]. The most commonly employed NSAIDs to this end are ketorolac, diclofenac, bromfenac, nepafenac and in some countries indomethacin [6–10]. Topical ocular administration of some drugs has been shown to produce systemic effects. For instance, topical 10% phenylephrine eyedrops increase transiently, but significantly, blood pressure and heart rate [11], while ophthalmic timolol may significantly reduce heart rate, especially during physical exercise [12]. Moreover, occasional severe systemic adverse events from topical ocular drugs have been reported, such as mental and cardiac disturbances after ocularly applied atropine eyedrops, or coma in an infant after ophthalmic brimonidine [13,14].

Cataract surgery is performed mainly in elderly patients [1], a population with a high incidence of ischemic cardiovascular events and/or of atrial fibrillation and venous thromboembolism, conditions that require lifelong antithrombotic therapy [15–17]. Systemically administered NSAIDs are contraindicated in patients on antithrombotic therapy for the enhanced bleeding and cardiovascular risk. In particular, some NSAIDs may enhance the anticoagulant activity of anti-vitamin K drugs through a pharmacokinetic interaction or through protein binding displacement, but their principal adverse interaction,

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enhancing the bleeding risk, is due to their ability to inhibit platelet function [18,19]. No data are available on the potential systemic effects of ocular administered NSAIDs, and in particular on their possible effects on platelet function.

Aim of our study was to assess the comparative effects of two commonly used NSAIDs, indomethacin and diclofenac, on circulating blood platelets in patients undergoing cataract surgery.

2. Methods

2.1. Study design

Patients candidate to cataract surgery were recruited at the Section of Ophthalmology of the Department of Surgical and Biomedical Sciences of the University of Perugia. Twenty patients were enrolled in a prospective, single-blind, randomized, parallel-groups trial. Inclusion criteria were: age above 18 years, planned cataract surgery and granting of informed, written consent. Exclusion criteria were: systemic use of NSAIDs, antiplatelet agents, oral anticoagulants, abnormal blood count, liver and/or kidney disease, pregnancy, intolerance to NSAIDs, inability to give informed consent.

The first 20 consecutive subjects who fulfilled the inclusion criteria at the preoperatory visit and who gave their informed consent were randomly assigned, according to a computer-generated sequence, to eyedrops of either Diclofenac 1 mg/ml (Voltaren Ofta, Théa Farma S.p.A., Italy) or Indomethacin 0.1 mg/ml (Indocollirio, Bausch & Lomb S.p.A., Italy). Blood for platelet function testing was drawn in the morning under fasting conditions, at the preoperative visit, before treatment with ocular eyedrops was started, and then on the day of surgery, after 3 days of eyedrop instillation 4 times a day, 15–20 min after the last medically-supervised eyedrop administration.

On the day of intervention patients followed the usual pre-operative procedures, i.e. their pupils were dilated by phenylephrine and tropicamide (Mydriasert, Théa Farma SpA, Milan, Italy) into the eyelid fornix about 60 min before surgery, and surgery was then performed according to the standard procedure with phacoemulsification and intraocular lens implantation. All the procedures were completed without intraoperative or postoperative complications.

The study was approved by the local Ethics Review Board (CEAS Umbria, prot. nr. 5928/15/V) and all subjects provided written informed consent.

3. Methods

Peripheral venous blood was collected in 3.2% sodium citrate tubes or in non-anticoagulated glass tubes. Laboratory investigators were blinded to treatment allocation.

Light transmission aggregometry (LTA) was studied in platelet-rich plasma (PRP) using arachidonic acid (AA) as an agonist, with an APACT 4 aggregometer (Alfa Wasserman, Milan, Italy) [20]. A concentration-response curve (*range* 0.1 to 1 mM) was generated to establish the half maximal effective concentration (EC_{50}), i.e. AA concentration inducing an aggregation amplitude half of maximal. PFA-100® (Dade-Behring, Deerfield, IL, USA) collagen/ADP (C/ADP) and collagen/epinephrine (C/EPI) cartridge closure times were also measured, as described [20,21]. The expression of P-selectin (CD62P) on the surface of circulating blood platelets was measured by flow cytometry (FC500, Beckman Coulter, Miami, Florida, USA), as previously reported [22]. Briefly, whole blood samples were incubated with saturating concentrations of anti-CD62P fluorescein isothiocyanate (FITC) and of an anti-CD41 phycoerythrin (PE)-labeled antibody as a platelet-specific marker. After 30 min of incubation in the dark, samples were diluted in PBS and analyzed. Platelets were identified by their forward scatter characteristics and by their positivity for CD41. Events in this region positive for FITC were considered to represent platelets expressing P-selectin and results are expressed as percentage of positive platelets. An appropriate isotype control was used to set non specific signal threshold (2%) [20,22].

Finally, TxB_2 was measured in the supernatant of platelets stimulated with arachidonic acid and in serum samples using an EIA (Cayman Chemical, Michigan, USA), as reported [23].

3.1. Statistical analysis

Because no previous reference data were available, we estimated that a sample size of 10 subjects per group could achieve 80% power to detect a mean of paired differences of AA-induced maximal amplitude of aggregation at LTA of 10% between the two groups, with an estimated standard deviation of differences of 8% and with a significance level (alpha) of 0.05 using a two-sided Wilcoxon test and assuming that data distribution was normal.

All data were tested for normal distribution with the D'Agostino Omnibus normality test and are presented as mean and standard error of the mean (S.E.M.), as appropriate. Categorical data were analyzed using the χ^2 test.

A p-value of < 0.05 was considered as statistically significant. All analyses were performed using MedCalc® v. 12.2 (MedCalc Software bvba).

Half-maximal effective concentrations (EC_{50s}) and related parameters were determined by nonlinear regression-sigmoidal dose response curves with variable slope function for 6 concentrations of AA. We used the EC50 shift function for comparison of multiple concentration response curves. Individual points on a curve were omitted if calculated as outliers by a separate nonlinear regression analysis (sigmoidal dose response) on individual curves by group (calculated with GraphPad Prism 5.01, La Jolla, CA, USA).

4. Results

A total of 20 patients were enrolled in the study (Group Diclofenac = 10, 5 females; Group Indomethacin = 10, 5 Females). Mean age was 73.5 ± 7.4 years (range 59–90); females were 50%; major cardiovascular risk factors were equally distributed in the two groups. Both systemic and topical ocular treatments did not differ between the two groups. Patient characteristics are reported in Table 1.

4.1. Ex vivo platelet activation

Baseline ex vivo platelet activation was not different between the two treatment groups.

AA-induced LTA was significantly inhibited after eyedrop therapy in the group treated with indomethacin, but not in the group treated with diclofenac. In particular, the EC_{50} for AA-induced platelet aggregation

Table 1
Patient characteristics at enrolment.

	Group diclofenac N = 10	Group indomethacin N = 10	p
Age (mean ± SD)	71.3 ± 7.2	76 ± 7.3	ns
Sex (% F)	50	50	ns
Comorbidities			
Hypertension (n)	4	5	ns
Type2 diabetes mellitus (n)	0	0	ns
Hypercholesterolemia (n)	0	1	ns
Smoker (n)	3	0	ns
Glaucoma (n)	3	1	ns
Treatments			
Antihypertensive agents (n)	4	5	ns
Proton pump inhibitors (n)	2	1	ns
Statins (n)	0	1	ns
Antidiabetic agents (n)	0	0	ns
Ocular antiglaucoma therapy (n)	2	1	ns

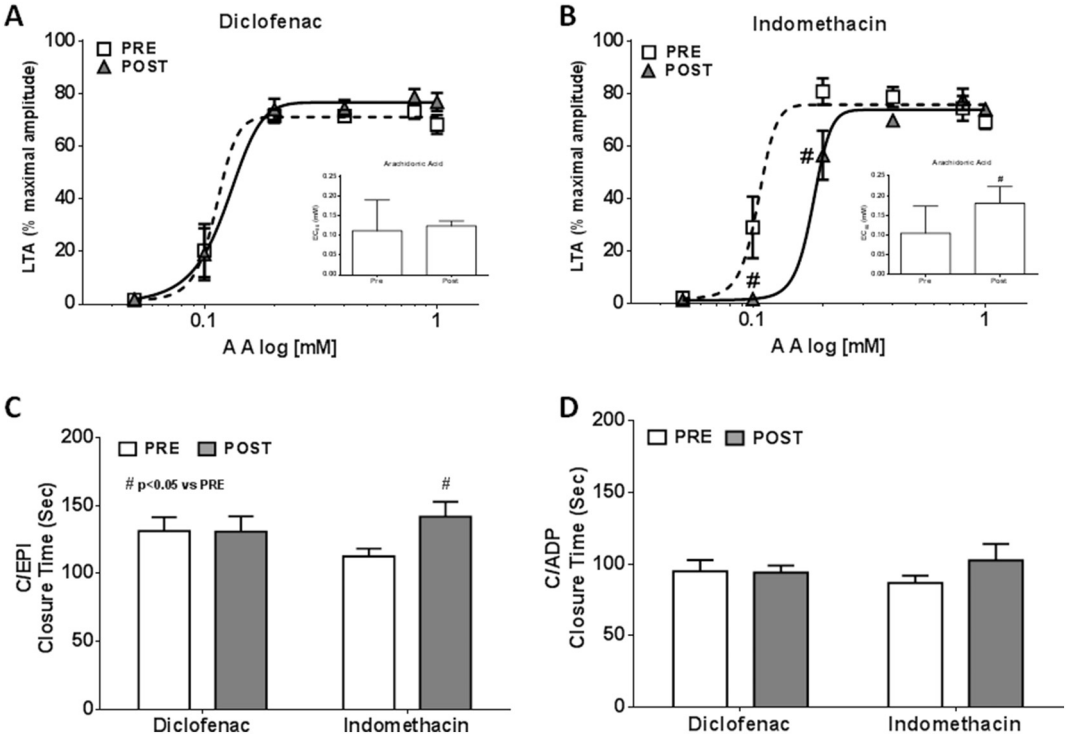

Fig. 1. Arachidonic Acid (AA)–induced platelet aggregation in diclofenac (A)- or Indomethacin (B)-treated subjects. Platelet rich plasma (PRP) was stimulated with increasing concentrations (0.1 to 1 mM) of AA and aggregation was followed for 5 min in samples collected prior and after 3 days of eyedrops. The insets show the EC50 for AA-induced platelet aggregation after diclofenac (A) or indomethacin (B) treatment. Squares = pre treatment; triangles = post treatment. # = $p < 0.05$ vs pre.

Effect of diclofenac or indomethacin eyedrop on the PFA 100® closure time. Indomethacin treatment induced a significant prolongation of the collagen-epinephrine (C/EPI) cartridge filter closure time (C) while the collagen-ADP (C/ADP) cartridge filter closure time (D) was not affected by either treatment. Empty column = pre treatment; grey column = post treatment. # = $p < 0.05$ vs pre.

was significantly shifted towards the right after indomethacin treatment (from 0.10 ± 0.07 mM to 0.18 ± 0.04 mM, $p = 0.001$), while no changes were observed after diclofenac treatment (Fig. 1A, B).

A significantly lower maximal amplitude of platelet aggregation was observed when platelets were stimulated with AA 0.1 mM (from 28.9 ± 11.7% to 1.4 ± 0.63%, $p = 0.032$) or AA 0.2 mM (80.7 ± 5.04% to 52.6 ± 12.4%, $p = 0.01$), after 3 days of indomethacin eyedrops, while no changes were observed after 3 days of diclofenac eyedrops (from 20.4 ± 10.1 to 19.0 ± 9.9%, p = ns, and from 71.6 ± 8.3% to 74.1 ± 12.7% p = ns).

A significant prolongation of the collagen-epinephrine (C/EPI) cartridge filter closure time was observed after indomethacin treatment (from 112 ± 5.7 to 171.8 ± 10.9 s, $p = 0.04$), but not after diclofenac treatment (from 131.2 ± 10.1 s. to 130.8 ± 11.1 s, p = ns) (Fig. 1C). On the contrary, the collagen-ADP (C/ADP) cartridge filter closure time was not affected by either treatment (Fig. 1D).

4.2. In vivo platelet activation

P-Selectin expression on circulating platelets at baseline did not differ between the two groups. P-selectin levels were significantly higher in the enrolled patients than in our standard healthy population [24] (17.5 + 1.9 vs 8 + 0.8%, $p < 0.05$), compatible with advanced age and concomitant cardiovascular risk factors in the cataract surgery population. Circulating platelet P-selectin was significantly lowered by indomethacin treatment (from 18.8 ± 1.0% to 9.1 ± 1.1%, $p = 0.01$), but not by diclofenac treatment (from 17.2 ± 0.9% to 13.0 ± 1.0%, p = ns) (Fig. 2A,B).

4.3. Tromboxane B_2

Serum tromboxane B_2 levels were similar in the two groups at baseline and no changes were observed after either treatment (Fig. 2C).

Thromboxane B_2 generation in the supernatant of AA (0.1 mM)-stimulated platelets was similar at baseline in the two treatment groups (0.38 ± 0.14 vs 0.42 ± 0.05 pmol/10^8 platelets, p = ns). Treatment with eyedrop indomethacin strikingly suppressed AA-induced TxB_2 generation (from 0.43 ± 0.06 to 0.04 ± 0.01 pmol/10^8 platelets, $p < 0.03$), while treatment with diclofenac did not modify it (Fig. 2D).

5. Discussion

Here we show that a short-term treatment with indomethacin eyedrops significantly inhibits platelet cyclooxygenase (COX-1) and impairs platelet aggregation and primary hemostasis.

In a double-blind, randomized trial the ocular administration of one drop of indomethacin collyrium 4 times a day for 3 days to patients undergoing cataract surgery inhibited AA-induced platelet aggregation and TxB_2 generation, prolonged PFA-100® C/EPI closure time and reduced the expression of P-selectin on circulating platelets while treatment with diclofenac collyrium at the same dose for the same period, did not affect them.

AA-induced platelet aggregation and TxB2 generation represent pharmacologic target-specific platelet function tests for NSAIDs [25]. The observation that only low AA-concentration induced, and not high concentration-induced, aggregation and TxB_2 generation were inhibited by indomethacin treatment is compatible with the reversible, competitive nature of the inhibition of COX-1 by indomethacin [26], and with the attainment of a relatively low systemic concentration of indomethacin [27].

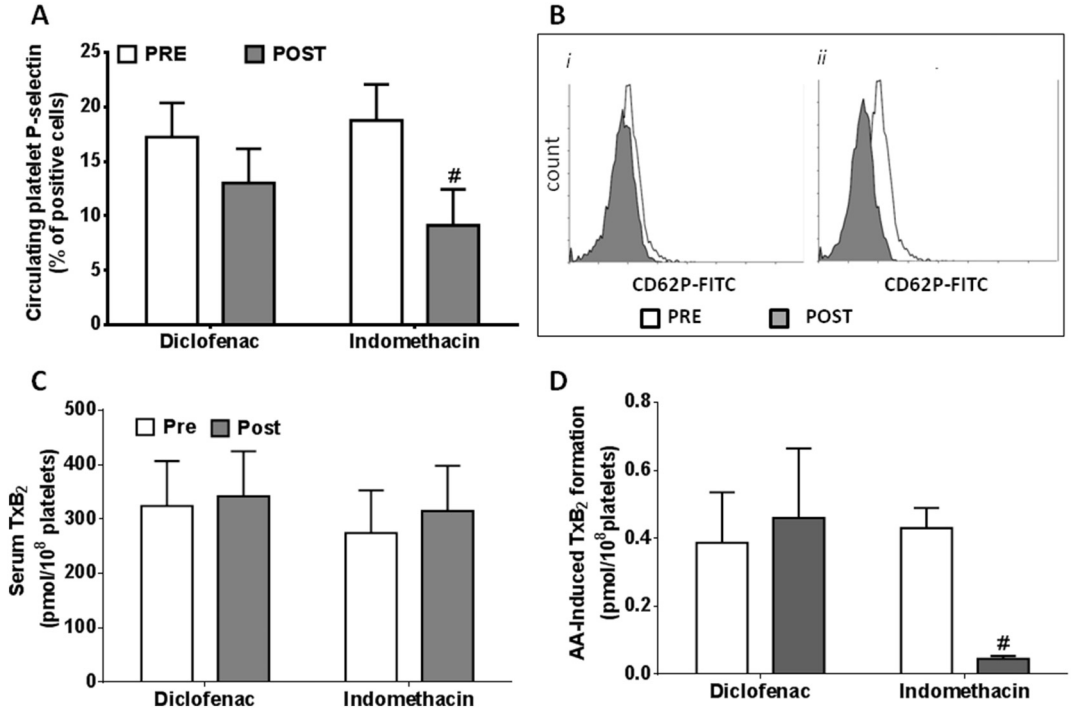

Fig. 2. Effect of diclofenac or indomethacin eyedrop on in vivo platelet activation. (A) Expression of P-selectin on the surface of circulating platelets was significantly inhibited by indomethacin treatment and not by diclofenac treatment. Empty column = pre treatment; grey column = post treatment. # = $p < 0.05$ vs pre. (B) Representative FC histogram of platelet P-selectin expression prior and after treatment with diclofenac (i) or indomethacin (ii).
Effect of diclofenac or indomethacin eyedrop on thromboxane B_2 levels. Serum tromboxane B_2 levels were similar in the two groups at baseline and no changes were observed after either treatment (C) while AA (0.1 mM)-induced TxB_2 generation was strikingly inhibited by treatment with eyedrop indomethacin but not by eyedrop diclofenac (D). Empty column = pre treatment; grey column = post treatment. # $p < 0.05$ vs Pre.

Treatment with indomethacin reduced also high-shear stress-induced platelet activation (PFA-100), a phenomenon of particular pathophysiologic relevance to in vivo thrombus formation and a surrogate marker of primary hemostasis [28,29]. On the other hand, circulating platelet P-selectin, a marker of in vivo platelet activation [20,30,31], was also suppressed, showing that indomethacin reduced significantly in vivo platelet activation.

The stronger systemic platelet inhibitory effect of topical indomethacin as compared with diclofenac is likely due to the higher COX-1/COX-2 selectivity ratio of the former NSAID (COX)-1/COX-2 ratio 3) compared to the latter (COX-1/COX-2 ratio 0.15) [32].

COX in platelets is represented mainly, if not exclusively, by COX-1 [33] while in inflamed tissue, like in the surgically-damaged conjunctiva, it is mainly COX-2 [32,34]. This may explain why diclofenac eyedrops are among the most effective topical NSAID for the treatment of anterior chamber inflammation [35] and in reducing vitreous prostaglandin E_2 levels [36] without simultaneously inducing any suppression of systemic platelet function.

An alternative explanation may be that systemic absorption by the conjunctival mucosa is lower for diclofenac that for indomethacin. However, this does not seem likely because in a comparative study assessing concentrations of NSAIDs and PGE_2 suppression in the vitreous cavity after eyedrop administration, it was shown that indomethacin and several aryl-acetic NSAIDs all penetrated the vitreous and lowered PGE_2 levels [36].

Although mild, the systemic impairment of platelet function observed after treatment with indomethacin eyedrops is biologically relevant because not only arachidonic acid-induced platelet aggregation, but also a surrogate parameter of primary hemostasis, like the PFA-100 [21,37] and even the expression of an activation marker on circulating platelets, like surface P-selectin [20,30–32], were significantly suppressed. Although very likely short-lived [38], due to the reversible nature of COX-1 inhibition by indomethacin, and relatively mild, the impairment of platelet function by indomethacin may enhance the bleeding risk when associated to oral anticoagulant therapy [39,40], especially in elderly subjects which are already at higher risk of hemorrhagic complications during chronic anticoagulation [40–44].

This pharmacologic interaction may thus be relevant in patients undergoing cataract surgery, a procedure carried out most frequently in elderly subjects, who are a population with a high prevalence of atrial fibrillation and venous thromboembolism, and thus often require long-term oral anticoagulation, and this is especially important considering that cataract surgery is usually carried out without interrupting oral anticoagulants [45,46]. Systemic effects of topical ocular drugs, including actions generating potential adverse events on the cardiovascular system, have been previously reported [11,12,47]. Therefore, caution should be taken in using ocular indomethacin in elderly patients on antithrombotic treatment and/or at high bleeding risk.

In conclusion, our study shows that a short course of topical ocular indomethacin impairs systemic platelet function and that this should be carefully considered in patients at enhanced risk of bleeding events, like elderly patients treated with oral anticoagulants.

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