In vivo modulation of the inflammatory response by nonsteroidal antiinflammatory drug-related compounds that trigger L-selectin shedding

Ada Herrera-García1, María Domínguez-Luis1, Mayte Arce-Franco1, Judith López-Fernández2, Manuel Feria3, Olga Barreiro4, Francisco Sánchez-Madrid4 and Federico Díaz-González1,5

1 Servicio de Reumatología, Hospital Universitario de Canarias, La Laguna, Spain
2 Servicio de Endocrinología y Nutrición, Hospital Universitario de Canarias, La Laguna, Spain
3 Departamento de Farmacología, Facultad de Medicina, Universidad de La Laguna, La Laguna, Spain
4 Servicio de Inmunología, Hospital Universitario de La Princesa, Madrid, Spain
5 Departamento de Medicina, Facultad de Medicina, Universidad de La Laguna, La Laguna, Spain

Diphenylamine-based nonsteroidal antiinflammatory drugs (NSAIDs) are able to cause in vitro the shedding of L-selectin. The aim of this work was to determine the physiologic relevance of L-selectin shedding in the antiinflammatory effect exerted by NSAIDs in vivo. Chemical compounds structurally related to NSAIDs — including diphenylamine, N-phenylantranilic acid (N-Ph), diphenylacetic acid — as well as the traditional NSAID indomethacin were studied using the zymosan air-pouch mouse model. Animals intramuscularly pretreated with indomethacin or N-Ph, but not with diphenylamine or diphenylacetic acid, showed a significant dose-dependent reduction in the number of neutrophils compared with untreated animals (N-Ph, IC50 = 6.7 mg/kg). Except for indomethacin, none of these compounds caused any significant reduction in cyclooxygenase-1 activity in vivo. In flow chamber experiments, N-Ph reduced the capability of human neutrophils to pass across the endothelial barrier by interfering with leukocyte rolling step on HUVEC. N-Ph, but not diphenylacetic acid, induced activation-independent L-selectin shedding in mouse neutrophils. Interestingly, N-Ph exerted an antiinflammatory effect similar to that of the anti-L-selectin blocking antibody Mel-14, although no additive action was observed when both compounds were combined. These data suggest that the L-selectin shedding induced by NSAIDs may be involved in the antiinflammatory action exerted by these compounds in clinical settings.

Keywords: Air-pouch mouse model · L-selectin · Nonsteroidal antiinflammatory drugs · N-phenylantranilic acid

Introduction

The recruitment of leukocytes into tissues during the inflammatory response is preceded by a highly coordinated sequence of adhesive events between flowing leukocytes and endothelial cells, a process known as the adhesion cascade. Members of three major
adhesion receptor families have been implicated in this cascade: selectins, integrins, and the immunoglobulin superfamily [1, 2]. In the field of inflammation, much effort is currently focused on developing antagonists of adhesion receptors, an approach known as antiadhesive therapy. This strategy is based on the assumption that if any one of the sequential steps of the adhesion cascade is inhibited, the inflammatory response is consequently suppressed or, at least, ameliorated [3, 4]. Although antiadhesive therapies targeting the major leukocyte integrins LFA-1, Mac-1, and VLA-4 have proven to be relatively successful in several human inflammatory disorders [4], the inhibition of selectins and their ligands has only proven beneficial in certain animal models of inflammation [5–8], with apparently only limited clinical effects on human inflammatory conditions [9].

Nonsteroidal antiinflammatory drugs (NSAIDs) are a heterogeneous group of therapeutic agents widely used for the symptomatic treatment of human inflammatory disorders. The blockade of cyclooxygenase (COX) has been widely accepted as the mechanistic action at work in these compounds [10]. During the last two decades, however, many groups have reported a number of nonprostaglandin-mediated antiinflammatory effects stemming from NSAIDs, suggesting that COX inhibition cannot be the only explanation for the antiinflammatory action observed with this group of therapeutic agents [11–16]. In this regard, it has been postulated that NSAIDs are able to interfere with the function of those adhesion molecules that participate in the adhesion cascade [17]. In neutrophils, for example, a group of NSAIDs induced the downregulation of L-selectin both in vitro [18, 19] and in vivo [18, 20]. Although the molecular mechanisms involved must still be fully clarified, in vitro experimental data suggest that the anti-L-selectin action of NSAIDs is prostaglandin independent, requires the presence of ADAM (a disintegrin and metalloprotease domain) 17, is related with the uncoupling of mitochondrial oxidative phosphorylation and that the NSAID structural core responsible for this effect appears to be diphenylamine [21].

Mouse models of inflammation have shown that, out of the three members of the selectin family (L-, P-, and E-selectin), L-selectin plays the major role in early recruitment of neutrophils at the inflammatory foci [22, 23]. L-selectin (CD62L), is constitutively expressed by most leukocytes, and it is enzymatically cleaved and released after cell activation both in vitro [24] and in vivo [25]. The shedding of L-selectin induced by such stimuli as PMA or TNF-α, stems from the processing of its ectodomain by members of the ADAM family of surface metalloproteases; specifically, ADAM17 [26] and ADAM8 [27]. It has been well established that L-selectin plays a key role in the inflammatory response mediating the rolling of leukocytes on endothelial cells [28]. However, whether the induction of L-selectin shedding plays an in vivo role in the antiinflammatory effects of NSAIDs, has not yet been established.

Here, using an animal model of acute inflammation, we evaluated the effects of several structurally related diphenylamine chemical compounds, which induce to varying extents the shedding of L-selectin in vitro. Our data indicate that the process of L-selectin shedding induced by diphenylamine-related compounds in vivo can interfere with the ability of neutrophils to accumulate at sites of inflammation. These data indicate that the induction of L-selectin shedding by NSAIDs might be responsible, at least in part, for the antiinflammatory effect that these compounds exert in vivo. The development of new chemical compounds specifically designed to target L-selectin might serve as a novel strategy for controlling the pathologic inflammatory response.

Results

In vivo effects of diphenylamine-based compounds on neutrophil recruitment to inflammatory foci

A group of NSAIDs, predominantly those with a diphenylamine-based chemical structure [21] are capable of inducing in vitro the rapid shedding of L-selectin in neutrophils [18]. In order to determine whether this effect on L-selectin might modulate the in vivo inflammatory response, we first decided to assess the biological role of several non-NSAID compounds structurally related to diphenylamine and indomethacin (Fig. 1A) in the air-pouch mouse model of acute inflammation. As described in Materials and Methods, mice were treated intramuscularly with 10 mg/kg of diphenylamine, N-phenylanthranilic acid (N-Ph) and diphenylacetic acid or indomethacin, a traditional NSAIDs used as control. Animals treated with N-Ph or indomethacin showed a significant reduction (~40%) in the number of cells that migrated to the air pouch with respect to control animals. However, diphenylacetic acid and diphenylamine did not show any significant effect (Fig. 1B). The effect of N-Ph on reducing neutrophil migration to the inflammatory foci in vivo showed a dose-dependent response with an IC50 of 6.7 mg/kg (Fig. 1C).

Although various processes are involved in the biological actions of NSAIDs, the inhibition of prostaglandin synthesis has been widely accepted as the main mechanism of action and the source of toxicity of these compounds [10]. To rule out the possibility that prostaglandin inhibition might play a role in the antiinflammatory action exerted by such diphenylamine-related compounds, we assayed the effects of indomethacin, diphenylamine, N-Ph, and diphenylacetic acids on COX-1 activity. The thromboxane B2 (TxB2) concentration induced by arachidonic acid was determined in platelets isolated from the same animals described in Fig. 1B, and was indicative of COX-1 reporter activity. As expected, indomethacin caused almost a complete inhibition of TxB2 release in mouse platelets, whereas neither diphenylamine nor diphenylacetic acid exerted any effects on COX activity in mouse platelets (Fig. 1D). Interestingly, N-Ph, a compound that, much like indomethacin, had caused robust inhibition of neutrophil recruitment in the air-pouch model, did not affect the basal production of TxB2. This observation ruled out the possibility that COX-1 inhibition plays a significant role in the antiinflammatory activity exerted by diphenylamine-related compounds in this animal model of inflammation.
Effects of diphenylamine-based compounds on the dynamics of neutrophil-endothelial cell interactions

The foregoing experiment showed that the systemic administration of N-Ph, a diphenylamine structure with a carboxylic radical in position 2 (Fig. 1A), was able to induce an antinflammatory effect equivalent to indomethacin, but via a prostaglandin-independent mechanism. Since the accumulation of white cells in inflamed tissues occurs as a consequence of adhesive interactions between activated endothelial cell and flowing leukocytes [29], we decided to study the effects of N-Ph and diphenylacetic acid both at human neutrophils and activated HUVECs sides in experiments investigating the dynamics of leukocyte-HUVEC interactions. When human neutrophils from healthy
volunteers were preincubated with N-Ph, but not with diphenylacetic acid, a significant reduction (~75%) in the number of cells that rolled on activated HUVECs was observed (Fig. 2A). In contrast, the treatment of TNF-α-activated HUVECs with either of these two compounds did not exert any significant effect on the number of rolling neutrophils with respect to controls. (Fig. 2A). When we assessed cell transmigration, the treatment of neutrophils with N-Ph, but not with diphenylacetic acid, reduced the percentage of cells that passed across HUVECs with respect to basal condition (Fig. 2B). Dreg 56, a blocking anti-human L-selectin mAb [30], inhibited about 80% the basal rolling of neutrophils, which demonstrated that under our experimental conditions, cell rolling is mainly an L-selectin-dependent phenomenon.

These data suggest that N-Ph reduces the capability of neutrophils to transmigrate across the endothelial barrier by interfering with the ability of neutrophils to roll on endothelial cells during the adhesion cascade. This effect of N-Ph was exerted selectively on neutrophils but not on HUVECs.

N-Ph induces the activation-independent downregulation of L-selectin in neutrophils in vivo

Since it is known that N-Ph induces in vitro a dose-dependent L-selectin downregulation in human neutrophils [21], our next step was to assess whether this compound exerted a similar effect in vivo. Animals were treated intramuscularly with 10 mg/kg of N-Ph or diphenylacetic acid. Neutrophils from animals treated with N-Ph expressed a significantly lower quantity of surface L-selectin than neutrophils from control animals (Fig. 3A). Accordingly, the plasma concentration of soluble L-selectin (sL-selectin) was significantly higher in animals treated with N-Ph than in controls (Fig. 3B).

However, when animals were treated with diphenylacetic acid, the L-selectin surface expression level was similar to controls animal (Fig. 3A). The basal expression of CD11b in mouse neutrophils, a β2 integrin that is upregulated on the neutrophil surface upon activation [31, 32], was not modified by any of these two compounds.
These experiments demonstrate that N-Ph is able to cause in vivo the shedding of L-selectin in neutrophils by an activation-independent mechanism.

N-Ph exerts its antiinflammatory action in vivo through the induction of L-selectin shedding

In order to determine to what extent the downregulation of L-selectin, as induced by N-Ph, might explain its antiinflammatory action in vivo, animals were injected intraperitoneally with either Mel-14 or an isotype-matching control antibody and then treated with or without N-Ph, as described in Materials and Methods. After 6 h, neutrophils from animals injected with Mel-14 showed, as previously described [33], two populations of cells expressing high and low levels of L-selectin, while control animals injected with the isotype mAb showed only the higher expressing neutrophil population (Fig. 4A). When N-Ph was administered intramuscularly to animals previously treated with either Mel-14 or an isotype control, their neutrophils only showed the population expressing low levels of L-selectin (Fig. 4A). Treatment with either Mel-14 or N-Ph did not significantly modify the basal expression of CD11b in mouse neutrophils (Fig. 4A). However, neutrophils from animals treated with Mel-14 showed a tendency to upregulate CD11b in the cell surface. When the plasma levels of sL-selectin was assessed in these animals, Mel-14 or N-Ph or both together induced, within a similar range, a significant increment in the concentration of sL-selectin with respect to animals treated with isotype mAb (Fig. 4B). In the air pouch model, Mel-14 caused a 50% reduction in the number of neutrophils recruited into the air bag. A similar effect was observed in animals treated with both N-Ph and an isotype-matching control antibody. Interestingly, animals treated simultaneously with Mel-14 and N-Ph did not show any significant additive reduction in the accumulation of neutrophils in the inflammatory foci with respect to animals treated with each one of them separately (Fig. 4C).

These results demonstrate that the capability of N-Ph to induce activation-independent L-selectin shedding can, by itself, cause a biologically relevant antiinflammatory effect in vivo.

Discussion

The most relevant findings of this study are: (i) NSAID-related chemical compounds based on diphenylamine are able to cause a biologically relevant antiinflammatory action in vivo; (ii) this effect is independent of prostaglandin synthesis inhibition and seems to be exclusively based on the activation-independent induction of L-selectin shedding in flowing neutrophils.

L-selectin is constitutively expressed on most leukocytes and, by recognizing glycosylated endothelial ligands, it plays a pivotal role in the initial interaction of leukocytes with the
endothelium, causing the rolling of neutrophils during the inflammatory response [34]. Upon activation, neutrophils downregulate L-selectin surface expression by shedding, thereby generating sL-selectin [28]. This soluble form of L-selectin has been detected in the plasma of normal human donors, and it has been suggested that it could be capable of retaining functional antiinflammatory activity [35]. Since L-selectin has been demonstrated to be essential in a number of experimental inflammatory conditions [5–8], several different strategies have been employed to modulate the inflammatory response by targeting L-selectin adhesive function. Such approaches have focused on the direct blockade of the interaction between L-selectin and its ligands by monoclonal antibodies, small molecules, aptamers, or soluble ligands [36]. However, antiinflammatory strategies aimed at mimicking the physiologic level of L-selectin shedding that occurs during the rolling phase of the adhesion cascade [28] have not yet been explored. The induction of L-selectin shedding would prevent neutrophil rolling, and consequently the inflammatory response, through a dual action: (i) by reducing the L-selectin surface expression in neutrophils and (ii) by facilitating the occupancy of endothelial L-selectin ligands by higher concentrations of sL-selectin in plasma. Most likely, this unexplored strategy could offer advantages, in terms of antiinflammatory potency, over the more limited effectiveness of a sole L-selectin-ligand blockade.

A group of NSAIDs are capable of shedding L-selectin without affecting neutrophil viability, activation, or expression levels of other surface molecules [18, 37]. It has been demonstrated that diphenylamine is the structural core responsible for this NSAID action, and that diphenylamine and its related compound N-Ph induce the shedding of L-selectin through a prostaglandin-independent mechanism [21]. We used these two in vitro features of diphenylamine-based compounds; specifically, the induction of L-selectin shedding and the absence of COX-1 inhibition activity, to determine whether the induction of L-selectin shedding by NSAIDs plays a role in the antiinflammatory action that these compounds exert in clinical settings. Our data demonstrate that the systemic administration of N-Ph, was able to induce in vivo a significant reduction in the accumulation of neutrophils in the inflammatory foci within a similar range as indomethacin. N-Ph exerted this effect with an IC50 of 6.7 mg/kg, a dose 100 times less than what
is toxic in rodents [38]. In contrast to indomethacin, the systemic administration of N-Ph did not affect COX-1 activity in the animals’ platelets. Consistent with this result, a previous report had described that this compound did not inhibit recombinant COX-1 or COX-2 [39]. These data demonstrated that N-Ph exerted an antiinflammatory effect in vivo via a prostaglandin-independent mechanism. In flow chamber experiments with human neutrophils and HUVECs, N-Ph was able to reduce the extravasation of neutrophils by reducing the L-selectin-mediated rolling capability of neutrophils. Moreover, N-Ph exerted this effect on neutrophils, but not on HUVECs suggesting that N-Ph exerts the rolling inhibition independently of the endothelial P- and E-selectin expression.

It has been demonstrated that both indomethacin and diclofenac, at therapeutic doses, induce the shedding of L-selectin in healthy volunteers [18, 20]. In mice, N-Ph caused a reduction on the neutrophil expression of L-selectin and an increase in the concentration of sL-selectin in plasma, which demonstrate that, in vivo, this compound was able to cause the shedding of L-selectin in neutrophils. Because L-selectin is rapidly downregulated from the neutrophil cell surface upon cell activation [24, 40], we also analyzed the activation state of neutrophils from animals treated with the different compounds by the expression of CD11b [31, 32]. Surface expression of CD11b in neutrophils from mice treated with N-Ph was similar to control animals, showing that this compound induced the shedding of L-selectin by an activation-independent mechanism in vivo.

Although our data demonstrated that N-Ph was able to simultaneously cause both an antiinflammatory effect and the shedding of L-selectin in neutrophils in an animal model of inflammation, the causal relationship between these two phenomena had not been proven. Remarkably, N-Ph caused a 50% reduction in the number of neutrophils recruited into the air bag, an effect in the same range that Mel-14, but without having an additive anti-inflammatory effect in animals also treated with Mel-14. It is therefore conceivable that the antiinflammatory capability of N-Ph was mainly due to the induction of L-selectin shedding in neutrophils. Animals treated with Mel-14 and N-Ph did not show a significant reduction in the accumulation of neutrophils in the inflammatory foci compared with those treated with either one of them separately. These results strongly suggest that the capability of N-Ph to induce activation-independent L-selectin shedding exerts a biologically relevant antiinflammatory effect in vivo. Interestingly, neutrophils from animal treated with Mel-14 showed a tendency to downregulate L-selectin and upregulate CD11b surface expression. Previous results has shown that cross-linking of L-selectin by mAb induces intracellular proinflammatory signals [41, 42], which may explain the changes caused by Mel-14 in L-selectin and CD11b expression in vivo.

Taking into account the fact that several NSAIDs, such as diclofenac, aeclofenac, flufenamic acid, or mefenamic acid cause in vitro the shedding of L-selectin in neutrophils within a similar range as N-Ph [21], the data presented in this study supports our contention that the anti-L-selectin action exerted by NSAIDs might account, at least in part, for their antiinflammatory properties. The development of new drugs based on the “skeleton structure” of diphenylamine could provide a breakthrough in therapies against inflammation. It is reasonable to think that this new family of antiinflammatory agents will show a better safety profile than the currently available NSAIDs, because their therapeutic action will be based on the induction of L-selectin shedding in neutrophils, rather than on prostaglandin synthesis inhibition.

### Materials and methods

#### Antibodies and reagents

The following monoclonal antibodies (mAbs) were used: Fluorescein (FITC) conjugate rat IgG2b anti mouse/human CD11b, clone M1/70 (eBioscience, San Diego, CA, USA), anti-human L-selectin blocking mAb, Dreg-56 (Becton Dickinson Bioscience, San Jose, CA), rat IgG2a anti-mouse L-selectin blocking mAb, Mel-14 (ImmunoTools, Friesoythe, Germany), mouse IgG2a isotype controls (ImmunoTools), and P3 × 63 myeloma cell supernatant as a negative control.

Dimethyl sulfoxide (DMSO), N-ph, diphenylamine, diphenylacetic acid, and indomethacin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Hank’s balanced salt solution (HBSS) was purchased from Lonza (Belgium), and the culture media (199 medium) from PAA Laboratories (Pasching, Austria).

#### Cell isolation and treatments

Mouse neutrophils were isolated from blood obtained by cardiac puncture after cervical dislocation, and were then subjected to Biocoll (Biochrom AG, Berlin, Germany) density-gradient centrifugation for 20 min at 1800 rpm, followed by sedimentation at 1 g in 1.3% (wt/vol) dextran (Sigma-Aldrich Chemical Co.) for 20 min at room temperature. The neutrophil-enriched fraction was further purified by hypotonic lysis of erythrocytes, which yielded a purity higher than 95%, as assessed by CD11b positivity in flow cytometry analysis. Human neutrophils were isolated from peripheral blood obtained by venipuncture from healthy donors following the same protocol. Human umbilical vein endothelial cells (HUVECs) were obtained as previously described [43]. HUVECs were cultured in a 5% CO₂-humidified atmosphere at 37°C in culture media (199 medium) supplemented with 1% HEPES (PA), 1% penicillin (PA), and 20% fetal calf serum (PAA). All in vitro experiments were carried out in 5 mL disposable polypropylene tubes (Falcon Labware, Oxnard, CA).

### Mouse air pouch model

Experiments were conducted in male CD-1 mice weighing between 24 and 30 g. All experimental protocols for animal usage were reviewed and approved by the Ethics Committee of the University of La Laguna.
The air pouch is a widely used mouse model of acute inflammation [44–46]. Briefly, 5 mL of sterile air was subcutaneously injected into the back of the experimental animal on day 0, and the pouch was reinforced with an additional 3 mL of air on day 3 to maintain the pouch cavity. Six days after the initial air injection, animals were treated intramuscularly with diphenylamine, diphenylacetic acid, N-Ph, or indomethacin at 10 mg/kg. All chemicals had been previously solubilized directly in DMSO at 500 mg/mL and fixed at a final concentration of 5 mg/mL in saline buffer. In some experiments, animals were injected intraperitoneally with 100 μg Mel-14 or with an isotype-matching control antibody 1 h prior to treatment with N-Ph at the dose indicated. Control animals were treated intramuscularly with 50 μL of a solution containing 1% DMSO in saline buffer. One hour after intramuscular treatments with the different compounds, air pouches were injected with 0.5 mL of 1% zymosan (Sigma-Aldrich Co.) to induce a local inflammation. Animals were maintained at room temperature. Four hours after zymosan administration, the animals were sacrificed by cervical dislocation and the pouch exudates were collected with 10 mL of cold saline buffer. The total number of leukocytes in the exudate fluid was assessed by flow cytometry in an Epics XL flow cytometer (Beckman Coulter, CA) using a known concentration of fluorescent beads (Becton Dickinson Bioscience) as control.

Flow cytometry analysis

Neutrophils isolated from the peripheral blood of mice treated with the different compounds, as described above, were incubated with Mel-14 or FITC anti-CD11b mAbs at 4 °C for 30 min. After washing in PBS, cells labelled with Mel-14 were incubated at 4 °C for 30 min in the presence of Alexa Fluor 488-labeled goat anti-rat IgG and IgM antibodies (Molecular Probes, Eugene, OR). Neutrophils from animals treated intraperitoneally with Mel-14 or with an isotype-matching control mAb were labeled directly after isolation with Alexa Fluor 488-labeled goat anti-rat antibody. After washing in cold saline buffer, at least 5 × 10⁵ cells from each sample were analyzed in an Epics XL flow cytometer and the data were presented using logarithmic scales. The fluorescence produced by the isotype-matching control mAbs was considered as the background. Because the fluorescence conditions varied from one experiment to the next, data were normalized to express the relative mean fluorescence intensity (rMFI), according to the following equation:

\[ rMFI = \frac{MFI_{\text{compound}} - MFI_{\text{negative control}}}{(MFI_{\text{medium}} - MFI_{\text{negative control}}) \times 100} \]

Platelet TxB2 release induced by arachidonic acid

In platelets, COX is a rate-limiting enzyme involved in the transformation of arachidonic acid into thromboxane A2, a potent platelet-aggregating agent that is rapidly transformed into the stable metabolite TxB2. The release of TxB2 induced by arachidonic acid was determined in platelets of animals treated with different compounds according to the following protocol: Platelets were isolated at room temperature from mice peripheral blood mixed with buffered sodium citrate (0.13 M) at a proportion of 10:1. Platelet-rich plasma was then prepared by centrifugation of whole citrated blood at 120 g for 10 min at 20 °C. platelet-rich plasma was treated with 1 mM arachidonic acid for 5 min at 37 °C. The reaction was quenched by the addition of ice-cold ethanol, and samples were centrifuged at 14 000 rpm for 5 min at room temperature. The ethanol of the supernatant was evaporated using a lyophilizer, and the pellet was resuspended in the immunoassay buffer. TxB2 concentration in supernatants was measured with a quantitative TxB2 immunoassay according to the manufacturer’s protocol (R&D Systems Europe, Abingdon, UK). Data are expressed as the total amount of TxB2 in pg/mL.

Mouse sL-selectin ELISA

Plasma from animals treated with the different compounds as described above was collected for sL-selectin quantification. The
Statistical analysis

Results are expressed as the arithmetic mean ± SD or SE of the mean as indicated. Wilcoxon-rank sum tests or Wilcoxon-matched pairs tests were used to determine significant differences as indicated. IC50 values were determined by variable-slope sigmoid function using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA).

Acknowledgments: This work was supported by grants from the Fondo de Investigaciones Sanitarias of Spain to F. D-G (FIS 09/02209). The work was also supported by Red de Inflamación y Enfermedades Reumáticas (RIER) del Instituto de Salud Carlos III. We are indebted to all members of the Rheumatology Service from Hospital Universitario de Canarias for their continuous support.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

References


References


Abbreviations: ADAM: a disintegrin and metalloprotease domain - NSAID: nonsteroidal antiinflammatory drugs - N-Ph: N-phenylanthranilic acid - TxB2: thromboxane B2

Full correspondence: Prof. Federico Diaz-González, c.Ofra s/n La Laguna 38320, Santa Cruz de Tenerife, Spain Fax: +34-922-646792 E-mail: federico.diaz.gonzalez@gmail.com

See accompanying Commentary: http://dx.doi.org/10.1002/eji.201243166

Accepted article online: 14/9/2012

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim www.eji-journal.eu