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Interaction of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) with Reactive Oxygen Species (ROS): Possible Biomedical Implications

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Additional information is available at the end of the chapter

Abstract

The present chapter deals on the interaction of nonsteroidal anti-inflammatory drugs (NSAIDs), diflunisal, indomethacin, meloxicam, tenoxicam and piroxicam with reactive oxygen species (ROS) photogenerated in aqueous solution by the vitamin riboflavin employed as a dye sensitizer. Simple techniques as substrate and oxygen consumption and more sophisticated time-resolved spectroscopic methods were employed for the kinetic and mechanistic evaluation of the deactivation of the *in situ* generated ROS singlet molecular oxygen ($O_2(^1\Delta_g)$), superoxide radical anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) by the mentioned NSAIDs. Results could be prudently extrapolated to a possible action of NSAIDs in the retardation or inhibition of neuroinflammatory disorders, in which oxidative agents such as ROS were found to be upregulated. Despite the potential benefit, some adverse effects in humans reported in relation with high doses of NSAIDs alert about the cares that have to be taken about their use.

Keywords: antioxidants, NSAIDs, photosensitization, riboflavin, ROS

1. Introduction

In the last decades, it has been a widespread use of an increasing number of chemical compounds with analgesic, antipyretic, and anti-inflammatory properties. In order to remark their differences with other group of medicines which presents known bad side effects, they were labeled as nonsteroidal anti-inflammatory drugs with the acronym NSAIDs [1–3].

At the same time, many neuroinflammatory mediators, including oxidative agents such as reactive oxygen species (ROS), were found to be upregulated in neurodegenerative disorders (ND) that affect human brain areas [4, 5]. This fact immediately allows the proposal of some kind of cause-effect link between the presence of ROS, oxidation processes, neuroinflammation, and ND pathogenesis [4, 5].

Oxidative stress is a process that occurs in early stages of ND and is considered an identifier mark for their detection as could be evaluated by DNA, RNA, lipids, and protein oxidation levels [6–8]. Simultaneously, several studies have observed an inverse correspondence between prolonged NSAID administration and the development of some ND in humans, (for review, see Ref. [9]). So, it is now accepted that NSAIDs could play a protective role on many ND and one of the reasons of the great interest for getting more insight into the elucidation of the pathways and mechanisms of the oxidative processes in which several NSAIDs and different ROS take part.

The present chapter will analyze the results presented in two relatively recent papers that have been dedicated to evaluate the possible action of some NSAIDs as protectors against ROS-mediated oxidation/deterioration of biological targets [10, 11]. Those research works are focused on NSAIDs from different chemical structure classes, one salicylic acid derivative, diflunisal (DFN), an indolic acid derivative, indomethacin (IMT) (**Figure 1**) and the enolic acid derivatives, oxicams, represented by meloxicam (MEL), tenoxicam (TEN) and piroxicam (PIR) (**Figure 2**).
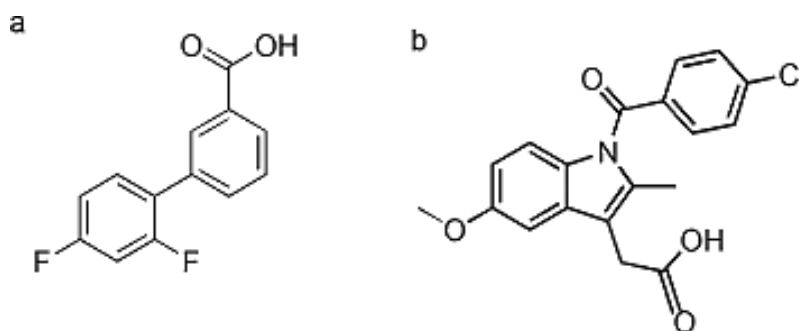

Figure 1. Chemical structures of **a:** 2′,4′-difluoro-4-hydroxyphenyl-3-carboxylic acid, diflunisal (DFN) and **b:** 2-{1′-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl} acetic acid, indomethacin (IMT).
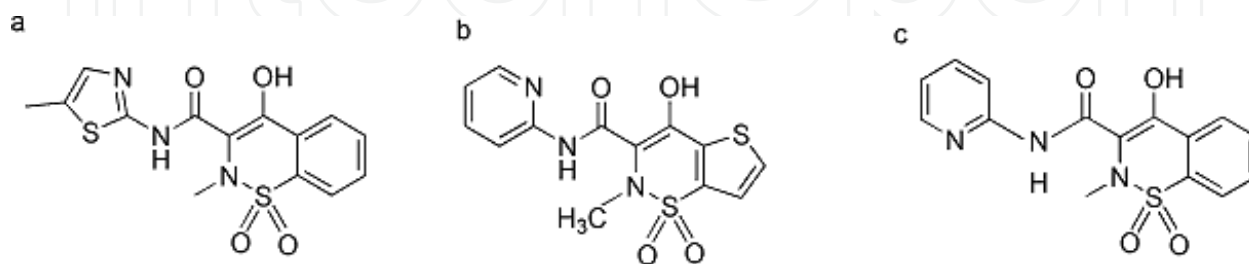

Figure 2. Chemical structures of **a:** [4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1 dioxide], meloxicam (MEL), **b:** [4-hydroxy-2-methyl-N-(pyridin-2-yl)-2H-thieno(2,3-e)-1,2 thiazine-3-carboxamide 1,1-dioxide], tenoxicam (TEN) and **c:** [4-hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1,2-benzothiazine-3-carboxamide 1,1-diox-ide], piroxicam (PIR).

2. Oxidation processes

Many compounds in the presence of oxygen and any electron donor can generate different ROS—by energy and/or electron transfer processes—like singlet molecular oxygen, $O_2(^1\Delta_g)$, superoxide radical anion $(O_2^{\bullet-})$ or hydrogen peroxide (H_2O_2) among others. An interesting example of those compounds is vitamin B2, riboflavin (Rf), a naturally occurring endogenous compound of singular importance, present in practically all living organisms. Rf absorbs energy in the wavelength range of visible light, being a well-known photosensitizer for oxidative processes [12, 13]. Upon selective absorption of energy, Rf is promoted from its ground state to electronically excited singlet state $(^1Rf^*)$ (Eq. (1)).
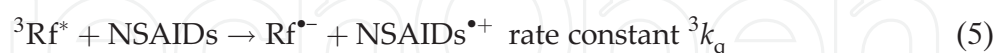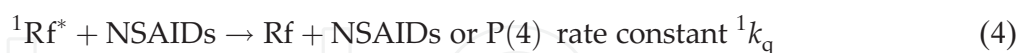
$$Rf + h\upsilon \to {}^1Rf^*$$
(1)

The generated $^1Rf^*$ can decay to the original ground state or produce the electronically excited triplet state $(^3Rf^*)$ (Eq. (2)).

$$^1Rf^* \to {}^3Rf^*$$
(2)

The $^3Rf^*$ may react with the ground state oxygen $(O_2(^3\Sigma_g^-))$ (Eq. (3)) to form superoxide radical anion $(O_2^{\bullet-})$, with a very low quantum yield (0.009) (Krishna, 1991).

$$^3Rf^*(^3\Sigma_g^-) \to Rf^{\bullet+} + O_2^{\bullet-}$$
(3)

In living organisms, a great number of biomolecules essential to life such as DNA, RNA, lipids, and proteins, can be oxidized by the generated ROS producing oxidative stress [6–8, 14]. Among other substrates, NSAIDs are compounds that can be oxidized in the presence of Rf-generated ROS and as shown can act as quenchers of electronically excited states of Rf (Eqs. (4) and (5)).

$$^1Rf^* + NSAIDs \to Rf + NSAIDs \text{ or } P(4) \text{ rate constant } {}^1k_q$$
(4)

$$^3Rf^* + NSAIDs \to Rf^{\bullet-} + NSAIDs^{\bullet+} \text{ rate constant } {}^3k_q$$
(5)

The protonation of $Rf^{\bullet-}$ at neutral pH can generate the species $RfH^{\bullet}$ ($pK_a = 8.3$), (Eq. (6)).

$$Rf^{\bullet-} + H^+ \rightleftarrows RfH^{\bullet}$$
(6)

Its bimolecular decay through a disproportionation reaction can yield the ground state of the vitamin and fully reduced Rf (Eq. (7)).

$$2RfH^{\bullet} \to Rf + RfH_2$$
(7)

The last product, in the presence of ground state oxygen, is reoxidized to Rf radical and superoxide radical anion $(O_2^{\bullet-})$ (Eq. (8)).

$$RfH_2 + O_2(^3\Sigma_g^-) \rightarrow RfH_2^{\bullet+} + O_2^{\bullet-} \quad \text{rate constant } k_8 \tag{8}$$

The electron transfer process, in Eq. (8) is relevant as a source of H_2O_2 (Eq. (9)), another important already-mentioned ROS.

$$RfH_2^{\bullet+} + O_2^{\bullet-} \rightarrow Rf + H_2O_2 \tag{9}$$

In parallel, the generated $O_2^{\bullet-}$ can chemically react with a substrate, according to Eqs. (10) and (11), respectively, illustrates the processes that occur with NSAIDs.

$$O_2^{\bullet-} \text{ NSAIDs} \rightarrow P(10) \quad \text{rate constant } k_{10} \tag{10}$$

$$H_2O_2 + \text{NSAIDs} \rightarrow P(11) \tag{11}$$

Another possible pathway for $^3Rf^*$ is the energy transfer reaction with $O_2(^3\Sigma_g^-)$ which generates $O_2(^1\Delta_g)$, with reported quantum yield of 0.49 in water [15] (Eq. (12)).

$$^3Rf^* + O_2(^3\Sigma_g^-) \rightarrow Rf + O_2(^1\Delta_g) \text{ rate constant } k_{ET} \tag{12}$$

The $O_2(^1\Delta_g)$ formed may be physically quenched either by the solvent (Eq. (13)).

$$O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-) \tag{13}$$

or by a substrate, as happens in the presence of NSAIDs (Eq. (14)).

$$O_2(^1\Delta_g) + \text{NSAIDs} \rightarrow O_2(^3\Sigma_g^-) + \text{NSAIDs} \text{ rate constant } k_q \tag{14}$$

Finally, Eq. (15) represents the main pathway of substrate disappearance in $O_2(^1\Delta_g)$ mediated processes.

$$O_2(^1\Delta_g) + \text{NSAIDs} \rightarrow P(15) \text{ rate constant } k_r \tag{15}$$

k_t being the overall rate constant for physical plus chemical quenching processes (Eq. (16)).

$$k_t = k_r + k_q \tag{16}$$

In order to get more insight into the behavior of NSAIDs toward Rf-generated ROS several *in vitro* experiments were performed.

2.1. Stationary photolysis: riboflavin-photosensitization

In complex biological structures, Rf and NSAIDs may occupy the same locations. Kinetic and mechanistic aspects of their mutual interaction constitute the crucial information for understanding the behavior of NSAIDs toward Rf-generated ROS and the potential *in vivo* consequences.

Using a home-made photolyzer, aerated neutral aqueous solutions of each of the following NSAIDs DFN, IMT, MEL, TEN, and PIR, were irradiated with the light of a 150W quartz-halogen lamp, in the presence of Rf as a sensitizer. All the NSAIDs used as substrates are transparent to visible light. Nevertheless, in order to assure that they do not absorb any incident radiation, a cut-off filter at 400 nm was employed. The processes were followed by the absorption spectra using a diode array spectrophotometer (Hewlett Packard 8452A). The light irradiation induced changes in the absorption spectra of the mixtures 0.05 mM DFN + 0.04 Rf (**Figure 3**), 0.05 mM IMT + 0.04 mM Rf (**Figure 3**, inset A) and 0.05 mM MEL + 0.04 mM Rf (**Figure 4**). The processes could be monitored from the absorbance decay at the respective absorption maxima for each substrate. In this way, the rates of sensitized photoxygenation for each NSAID were determined.

In parallel experiments, using a specific oxygen electrode (Orion 97-08) the oxygen concentration was measured during irradiation of the same mixtures in aqueous solutions in a closed Pyrex cell [10]. Under these conditions, all the NSAIDs under study showed oxygen consumption. Regarding the oxicams family, TEN and PIR presented the lowest rate of oxygen consumption. It was a little bit higher for MEL (**Figure 4**, inset B). In the corresponding set for DFN and IMT, the rate of oxygen uptake was significantly higher for the latter (**Figure 3**, inset B).
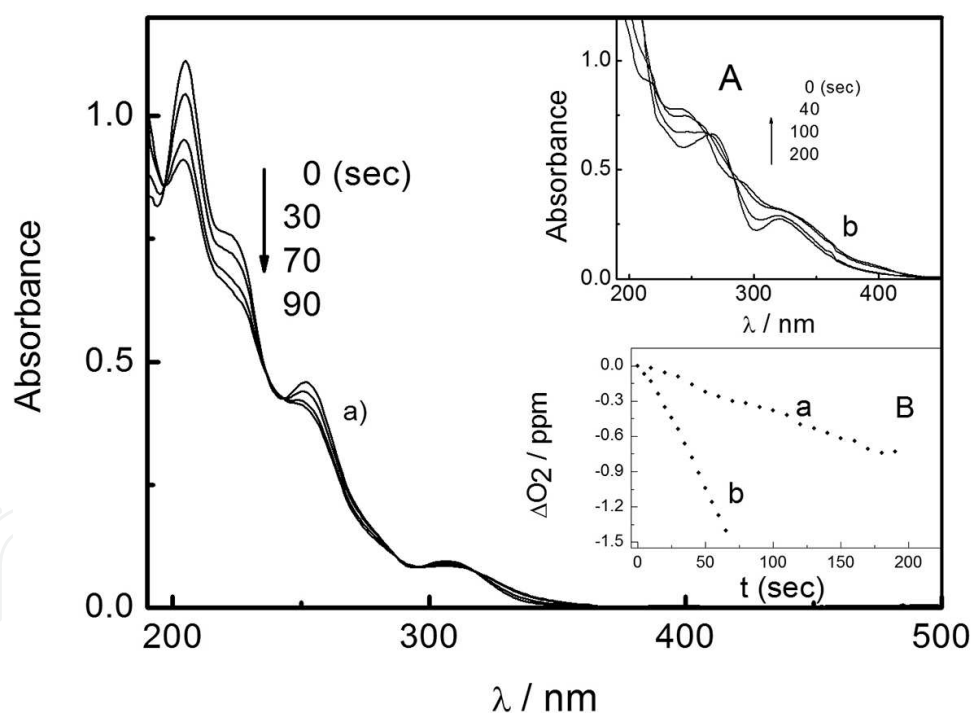

Figure 3. Changes in UV-vis absorption spectra of a pH 7 aqueous solution of 0.05 mM DFN plus 0.04 mM Rf upon photoirradiation taken vs. a 0.04 mM Rf aqueous solution (spectrum **a**). Cut-off 400 nm interference filter, under air-saturated conditions. Numbers on the spectra represent photoirradiation time in seconds. (**Inset A**) Changes in UV-vis absorption spectrum of a pH 7 aqueous solution of 0.05 mM IMT plus 0.04 mM Rf upon photoirradiation taken vs. a 0.04 mM Rf aqueous solution (spectrum **b**). Cut-off 400 nm interference filter, under air-saturated conditions. Numbers on the spectra represent photoirradiation time in seconds. (**Inset B**) Oxygen consumption vs. photoirradiation time in pH 7 aerated aqueous solutions for the systems: a: Rf (A_{446} = 0.46) plus DFN (0.4 mM); b: Rf (A_{446} = 0.46) plus ITM (0.4 mM). Reprinted from Purpora et al. [10], © (2013), with permission from The American Society of Photobiology, a Wiley Company, John Wiley & Sons, Inc.
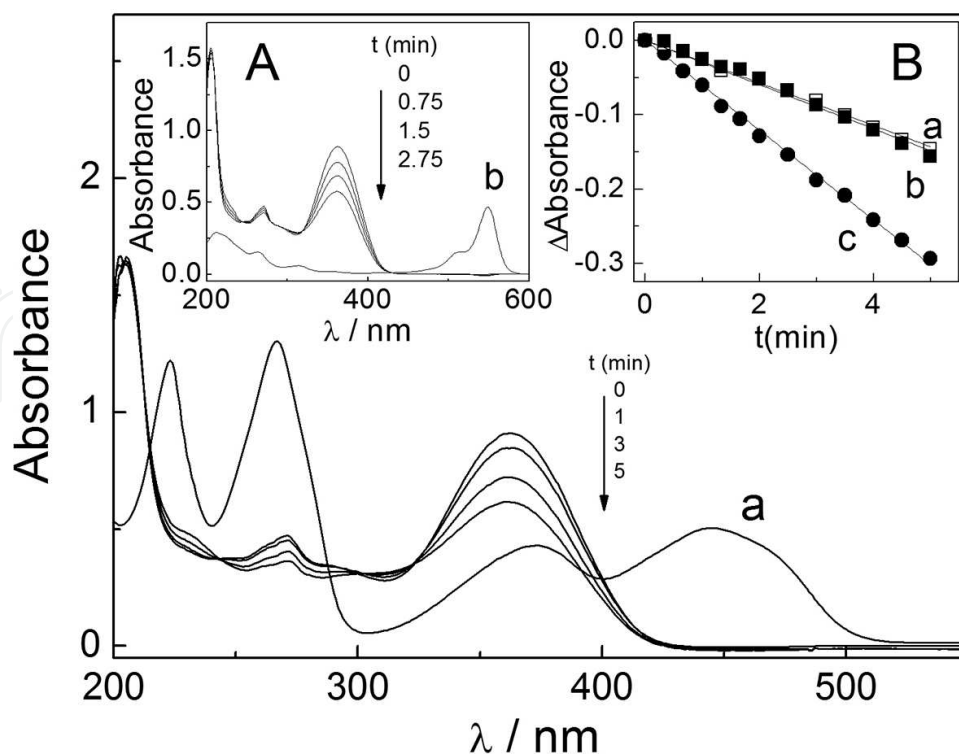
Figure 4. Changes in UV-vis absorption spectra of aqueous solution of 0.05 mM MEL plus 0.05 mM Rf upon photoirradiation taken vs. 0.05 mM Rf aqueous solution (spectrum **a**). Cut-off 450 nm interference filter, under air-saturated conditions. Numbers on the spectra represent photoirradiation time in minutes. (**Inset A**) Changes in UV-vis absorption spectra of aqueous solution of 0.05 mM MEL plus 0.05 mM RB upon photoirradiation taken vs. 0.05 mM RB aqueous solution (spectrum **b**). Cut-off 450 nm interference filter, under air-saturated conditions. Numbers on the spectra represent photoirradiation time in minutes. (**Inset B**) Oxygen consumption vs. photoirradiation time under air saturated conditions for the systems: a: Rf 0.05 mM plus TEN (0.5 mM) in MeOH-H$_2$O (buffer pH 7) 1:1 v/v; b: Rf 0.05 mM plus 0.5 mM *PIR* in MeOH-H$_2$O (buffer pH 7) 1:1 v/v; c: Rf 0.05 mM plus 0.5 mM MEL in aqueous buffer pH 7. $\lambda_{irr} > 480$ nm, cut-off filter. Reprinted from Ferrari et al. [11], © (2015), with permission from Elsevier B.V.

From all these preliminary findings, we assume that the transformations in NSAIDs can be attributed to interactions with electronically excited states of Rf with the possible participation of photogenerated ROS.

2.1.1. Kinetics and mechanism

The xantenic dye Rose Bengal (RB) is one of the most frequently employed photosensitizers that exclusively generate O$_2$($^1\Delta_g$), with a quantum yield of 0.7 in aqueous media [15, 16]. So, experiments performed in the presence of RB involved possible O$_2$($^1\Delta_g$)-mediated oxidation of NSAIDs. In this case, eventual interferences of other ROS that could be generated by Rf were avoided. Comparing the rates of substrate consumption by Rf – photosensitization with those in the presence of RB it was possible to elucidate the relevance of O$_2$($^1\Delta_g$) in relation to other ROS also generated by Rf.

The combination of stationary and time-resolved experiments unambiguously demonstrates the participation of O$_2$($^1\Delta_g$) in NSAIDs' photooxidation processes. Using time-resolved phosphorescence detection (TRPD) [17], the overall quenching rate constant of O$_2$($^1\Delta_g$) by NSAIDs,

NSAID	$^1k_q \times 10^{10}$ (M^{-1}s^{-1})	$^3k_q \times 10^9$ (M^{-1}s^{-1})	$k_t \times 10^8$ (M^{-1}s^{-1})	$k_r \times 10^8$ (M^{-1}s^{-1})
IMT	0.89 ± 0.06 (a)	1.8 ± 0.3	2.6 ± 0.2	2.7 ± 0.2
DFN	0.90 ± 0.05 (a)	2.1 ± 0.5	1.7 ± 0.3	0.19 ± 0.1
MEL	2.84 ± 0.12	1.5 ± 0.3	1.15 ± 0.06 (b)	0.73 ± 0.04
TEN	0.88 ± 0.04	1.9 ± 0.4	1.00 ± 0.07 (c) (1.1) (d)	0.50 ± 0.03 (0.61) (d)
PIR	2.30 ± 0.06	1.7 ± 0.3	0.49 ± 0.05 (c)	0.48 ± 0.06 (1.6) (e)

(a) In MeOH; (b) in D$_2$O, pH 7; (c) in MeOH-D$_2$O (pD 7) 1:1 v/v; (d) in dioxane-water (molar fraction of water = 0.91) *Source*: [18]; (e) in MeCN *Source*: [19].

Table 1. Values for the rate constants for the interactions of each NSAID by quenching with electronically excited singlet (^1k_q), and triplet (^3k_q) of riboflavin; overall rate constants (k_t) **and reactive** (k_r) for the interaction of $O_2(^1\Delta_g)$ with each NSAID.

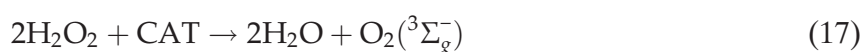| NSAID	k_r/k_t	RR$_{Rf}$	RR$_{RB}$
IMT	~1	1	1
DFN	0.11	0.26	0.07
MEL	0.63	1	1
TEN	0.52	0.48	0.68
PIR	~1.00	0.47	0.67

Source: [10, 11].

Table 2. Values for the ratio of the reactive and overall rates k_r/k_t, and relative rates of each NSAID consumption upon Rf (RR$_{Rf}$) and RB (RR$_{RB}$) photosensitization.
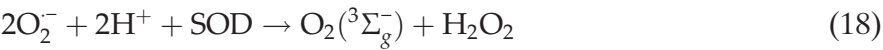
k_t (**Table 1**) was determined. Hence, k_t in the order of 10^7 M^{-1}s^{-1} allows the consideration of these substrates as good quenchers of $O_2(^1\Delta_g)$. The k_r/k_t ratio accounts for the fraction of the overall quenching of $O_2(^1\Delta_g)$ that produces chemical transformation in the substrate (**Table 2**). Low k_r/k_t values denote that $O_2(^1\Delta_g)$ removal will proceed without a significant loss of the present NSAIDs, which act as a scavenger [18, 19].

2.2. Interaction of NSAIDs with photogenerated ROS

Some compounds that are specific ROS quenchers have been used to elucidate which species are effectively involved in a given oxidative event [20, 21]. Catalase from bovine liver (CAT) reacts with H$_2$O$_2$, so the photodegradation via process in Eq. (11) is inhibited due to the process represented by Eq. (17).

$$2H_2O_2 + CAT \rightarrow 2H_2O + O_2(^3\Sigma_g^-) \tag{17}$$

The enzyme superoxide dismutase from bovine erythrocytes (SOD) dismutates the species O$_2^{\bullet-}$, as shown by Eq. (18).

$$2O_2^- + 2H^+ + SOD \rightarrow O_2(^3\Sigma_g^-) + H_2O_2 \qquad (18)$$

Meanwhile, sodium azide (NaN$_3$) is a known physical quencher of O$_2$($^1\Delta_g$), with a reported rate constant k_q of 4.5×10^8 M^{-1}s^{-1} in water at pH 7 (Eq. (14) with NaN$_3$ instead of NSAIDs) [22]. Several oxygen consumption experiments of NSAIDs upon Rf-photosensitization were performed adding each of these specific ROS interceptors. With DFN or IMT solutions different extent of decrease in the rates of oxygen consumption were observed upon using any of these three quenchers. This fact confirms a significant participation of O$_2$($^1\Delta_g$) in the degradation of
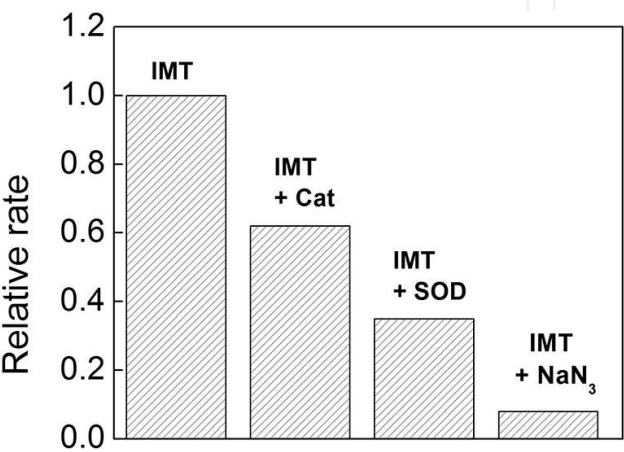

Figure 5. Bar diagrams for the relative rates of oxygen consumption with aqueous solutions pH 7, 0.5 mM IMT plus Rf (A$_{445}$ = 0.5) as function of photoirradiation time (cut-off 400 nm): ITM: alone; IMT + CAT: in the presence of 1µg mL^{-1} CAT; IMT + SOD: in the presence of 1µg mL^{-1} SOD; IMT + NaN$_3$: in the presence of 1 mM NaN$_3$. Reprinted from Purpora et al. [10], © (2013), with permission from The American Society of Photobiology, a Wiley Company, John Wiley & Sons, Inc.
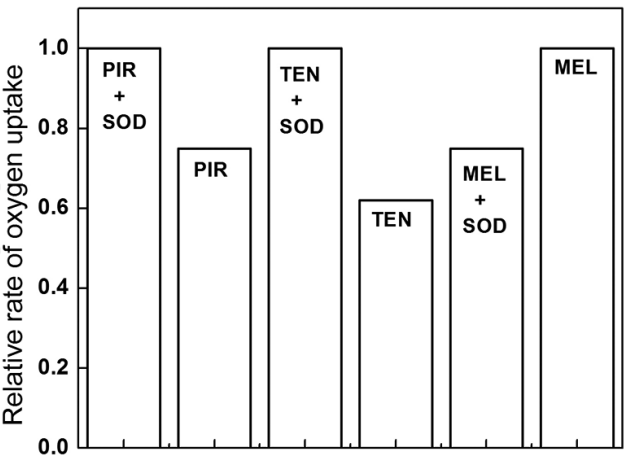

Figure 6. Bar diagrams for the relative rates of oxygen consumption upon photoirradiation as function of photoirration time (cut-off filter 400 nm) in the presence of Rf 0.05 mM with the following solutions: 0.5 mM PIR in MeOH-H$_2$O (buffer pH 7) 1:1 v/v plus 1 µg mL^{-1} SOD; 0.5 mM PIR in MeOH-H$_2$O (buffer pH 7) 1:1 v/v; 0.5 mM TEN in MeOH-H$_2$O (buffer pH 7) 1:1 v/v plus 1 µg mL^{-1} SOD; 0.5 mM TEN in MeOH-H$_2$O (buffer pH 7) 1:1 v/v; 0.5 mM MEL plus 1 µg mL^{-1} SOD in aqueous buffer pH 7; 0.5 mM MEL in aqueous buffer pH 7. Reprinted from Ferrari et al. [11], © (2015), with permission from Elsevier B.V.

the analgesics DFN and IMT, in which also $O_2^{\bullet-}$ and H_2O_2 take part. Bar diagram of the relative rates illustrates the results obtained with IMT solutions in the presence of each specific quencher (**Figure 5**); DFN solutions presented similar qualitative results.

Similar experiments were performed using solutions 0.5 mM of the three oxicams and NaN_3 NaN$_3$ or SOD. The participation of $O_2(^1\Delta_g)$ in the oxidation processes of these NSAIDs was revealed by the lower rates of oxygen uptake (**Figure 6**). As in the previous cases, for MEL the presence of SOD produced a decrease in the rates of oxygen consumption. Meanwhile for TEN and PIR it was the other way around. This fact can be due to the participation of $O_2^{\bullet-}$ with different mechanistic roles. The regeneration of $O_2(^3\Sigma_g^-)$ (Eq. (18)) at expenses of $O_2^{\bullet-}$ increases the $O_2(^1\Delta_g)$ leading to the detected rates increased.

3. Photoprotective effect of NSAIDs toward amino acids and peptides oxidation

In order to evaluate an eventual antioxidant/protective effect of NSAIDs towards biologically relevant substrates, amino acids (AA) and peptides may be employed as typical oxidizable targets in a proteinaceous medium.

Tryptophan (Trp) and tyrosine (Tyr) are AAs that can be affected by photo-damages through photodynamic activity [23, 24]. They are known quenchers of $^3Rf^*$ with ^3k_q of $2.5\times10^9 M^{-1}s^{-1}$ and $1.0\times10^9 M^{-1}s^{-1}$, respectively [13]. In order to evaluate the eventual protective effect of NSAIDs against photooxidation, Rf-photosensitized experiments were performed using each of these AA and the oxicam PIR. For comparative purposes, the trials were also performed replacing Rf by RB which ensures that the prevalent oxidation process is due to $O_2(^1\Delta_g)$. As a measure of the global photooxidative process, the rates of oxygen consumption were determined in each trial monitoring up to 10% conversion of the substrate under study.

PIR and Trp, as isolated substrates, are efficient $O_2(^1\Delta_g)$ chemical scavengers. Their k_r values are virtually identical, while the k_r/k_t relationship presents also very similar values. For the interaction Trp-$O_2(^1\Delta_g)$ it has been reported the rate constant values $k_t = 7.2\times10^7 M^{-1}s^{-1}$. and $k_t = 4.7\times10^7 M^{-1}s^{-1}$.[13, 25]. Using RB as the sensitizer, the rates of oxygen uptake for the mixture PIR + Trp were approximately equal to the rates of PIR and Trp individually considered, which may be due to the fact that they react through a pure $O_2(^1\Delta_g)$-mediated process (**Figure 7**). Employing Rf as the photosensitizer, the mixture PIR + Trp presented a rate of oxygen consumption significantly lower than the addition of the respective rates for each substrate. A possible explanation is that both compounds present a high ^3k_q value, so the simultaneous action of them may decrease the $O_2(^1\Delta_g)$ concentration leading to the lower rate observed with the presence of the mixture.

In neutral pH, Tyr is present in a very low reactive form. The interaction Tyr with $O_2(^1\Delta_g)$ mostly operates by physical deactivation of the ROS with a reported rate constant value
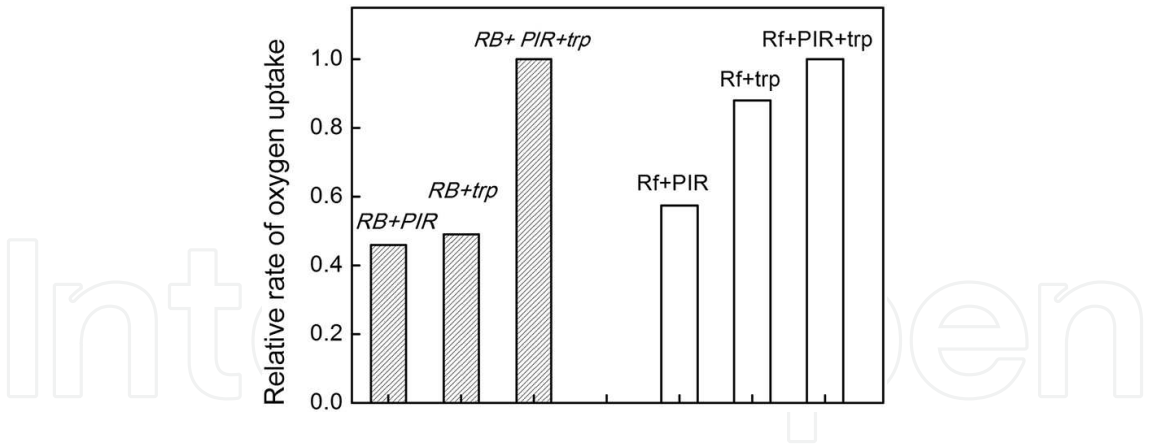
Figure 7. Bar diagrams for the relative rates of oxygen consumption upon RB (A_{560} = 0.4) photosensitization in pH 7 buffered aqueous solution by: 0.5 mM PIR; 0.5 mM Trp; 0.5 mM PIR plus 0.5 mM Trp. And upon Rf (A_{445} = 0.5) photosensitization in pH 7 buffered aqueous solution by: 0.5 mM PIR; 0.5 mM Trp; 0.5 mM PIR plus 0.5 mM Trp. Reprinted from Ferrari et al. [11], © (2015), with permission from Elsevier B.V.

$k_t = 1.5 \times 10^7 \mathrm{M}^{-1}\mathrm{s}^{-1}$ [22, 26]. The very low k_r/k_t may be due to the clear decrease in the rate of oxygen uptake by the mixture PIR + Tyr as compared to the one for the isolated PIR with RB as the photosensitizer. (**Figure 8**) With Rf as a sensitizer, the corresponding rates for PIR alone and the one for the mixture are practically equal.

A relevant result was that PIR in the presence of Rf showed an interesting degree of protection against Trp or Tyr oxidation by the *in situ*-photogenerated ROS. This fact has been revealed by
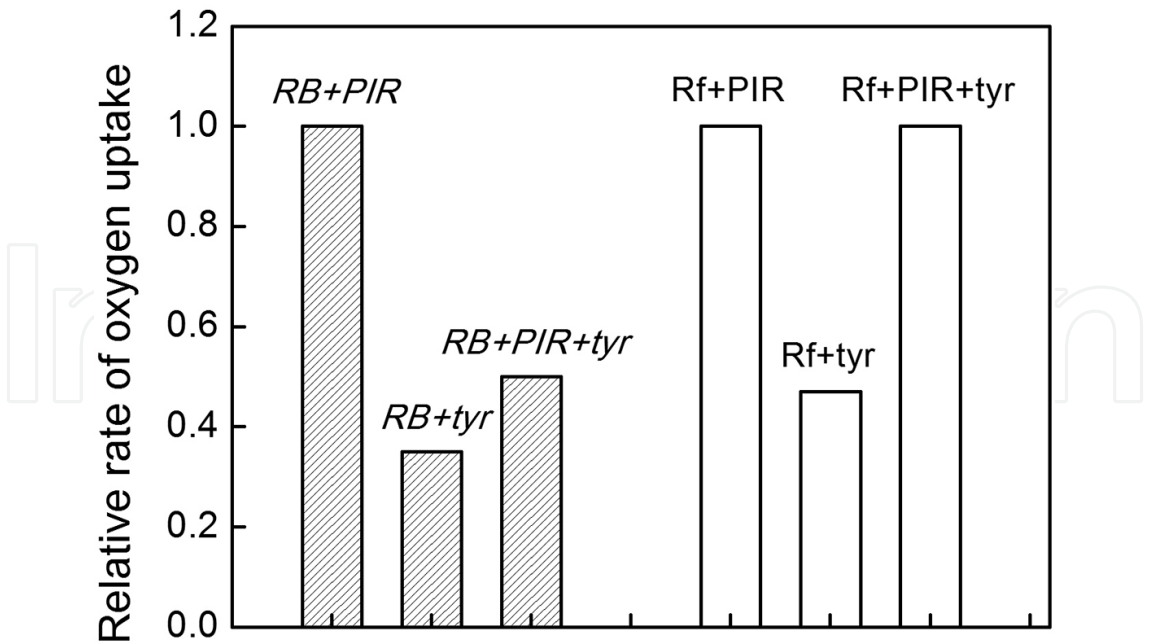

Figure 8. Bar diagrams for the relative rates of oxygen consumption upon RB (A_{560} = 0.4) photosensitization in pH 7 buffered aqueous solution by: 0.5 mM PIR; 0.5 mM Tyr; 0.5 mM PIR plus 0.5 mM Tyr. And upon Rf (A_{445} = 0.5) photosensitization in pH 7 buffered aqueous solution by: 0.5 mM PIR; 0.5 mM Tyr; 0.5 mM PIR plus 0.5 mM Tyr. Reprinted from Ferrari et al. [11], © (2015), with permission from Elsevier B.V.

the lower rates of oxygen consumption of the mixture oxicam-AA as compared to the ones for the individual substrates.

The dipeptide Trp-Tyr in a 0.5 mM aqueous solution was employed as a biologically relevant model compound, with RB or Rf as photosensitizers and IMT or DFN as potential photo-protective substrates. The $O_2(^1\Delta_g)$- mediated process of Trp-Tyr could be studied using RB alone. Its rate constant value $k_r = 5.9 \times 10^7 M^{-1} s^{-1}$ had already been reported [24]. The comparison of the relative rates of oxygen consumption in the presence and in the absence of 0.5 mM IMT showed that the value for the mixture Trp-Tyr + IMT was close to the simple addition of the respective individual rates (**Figure 9**).
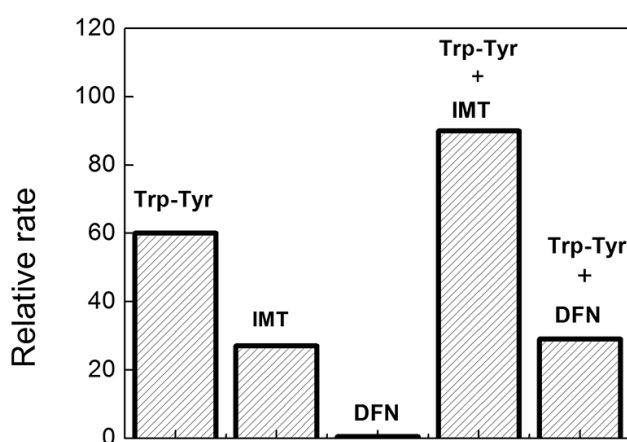

Figure 9. Bar diagram for the relative rates of oxygen consumption upon RB (A_{560} = 0.4) photosensitization in pH 7 buffered aqueous solution of: 0.5 mM Trp-Tyr; 0.5 mM IMT; 0.5 mM DFN; 0.5 mM Trp-Tyr plus 0.5 IMT; 0.5 mM Trp-Tyr plus 0.5 DFN. Reprinted from Purpora et al. [10], © (2013), with permission from The American Society of Photobiology, a Wiley Company, John Wiley & Sons, Inc.

Figure 10. Bar diagram for the relative rates of oxygen consumption upon Rf (A_{445} = 0.5) photosensitization in pH 7 buffered aqueous solution of: 0.5 mM Trp-Tyr; 0.5 mM IMT; 0.5 mM DFN; 0.5 mM Trp-Tyr plus 0.5 IMT; 0.5 mM Trp-Tyr plus 0.5 DFN. Reprinted from Purpora et al. [10], © (2013), with permission from The American Society of Photobiology, a Wiley Company, John Wiley & Sons, Inc.
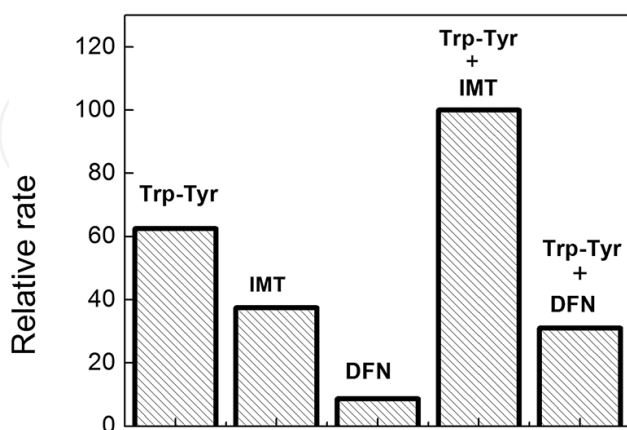
Meanwhile, the rate for the mixture Trp-Tyr + DFN decreased more than 50% of the one for the isolated dipeptide. Upon Rf-sensitization, similar results were obtained for DFN and IMT (**Figure 10**). This fact suggested that the photoxidation occurs mainly by reaction with the Rf-photogenerated $O_2(^1\Delta_g)$.

4. Conclusions

The results presented for the NSAIDs under study pointed out their efficiency as quenchers of photogenerated $O_2(^1\Delta_g)$. In Rf-photosensitized processes the dominant mechanism is the $O_2(^1\Delta_g)$-mediated, but also other ROS can be intercepted by most of them. The experiments here detailed showed that DFN and IMT can interact with H_2O_2 and O_2^- whereas MEL is an effective quencher for the latter Rf-photogenerated species.

DNF could be considered as an ideal scavenger of $O_2(^1\Delta_g)$, as the oxidative process occurs by a physical mechanism without significant self-degradation of this NSAID. In the case of IMT or oxicams, their protective effect decline along the time. The reason is that these scavengers can also be targets of the oxidation ROS-mediated processes. Even though, the *in vivo* antioxidant effectiveness would be warranted by daily and prolonged intake. Generally, that is the form of administration in which these analgesics are employed in the treatment of serious detrimental inflammatory illness or chronic pains.

Based on the discussed results, the NSAIDs studied herein present, in principle, promising properties for medicinal use as bio-antioxidants against *in situ* generated ROS. Nevertheless, great care must be taken because at the same time different negative effects in the human body have been reported [27, 28]. The literature on this topic, in most cases, only mentions rare but possible gastrointestinal adverse effects [29]. In the case of DFN, the reported side effects are not so dramatic, but IMT and MEL have been connected to the pathogenesis of gastric and intestinal mucosal lesions with participation of ROS [30–32]. Those undesired effects must be thoroughly taken into account mainly because of the relative high doses necessary with some of them in order to guarantee the replenishment, ensuring the antioxidant effectiveness against ROS activity.

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