Chemistry Research Journal, 2019, 4(1):6-11

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Metal chelating and reactive oxygen species scavenging properties of celecoxib (a COX-2 inhibitor) *in vitro*

Eboh Abraham Sisein[1], Ere Diepreye[2]

[1]Biochemistry Department, Niger Delta University, Bayelsa State, Nigeria
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta, University, Bayelsa State, Nigeria

Abstract Assays of drugs (e.g celecoxib) as an antioxidants are necessary because free radical productions in rheumathoid and other diseases are now generally accepted. The objective of the study was to investigate the free radical scavenging effect of celecoxib against H_2O_2, $NO^{\bullet}$, $O_2^{\bullet-}$, •OH and metal chelation. Arthritis is an inflammation of joints due to infectious, oxidants usually accompanied with pain, swelling, and stiffness of joints. All the assays were based on standard spectrophotometric methods. The results of celecoxib at 100 µM scavenged H_2O_2 at 43.16 ± 3.12 %, $O_2^{\bullet-}$ at 33.23 ± 0.78 %, Fe^{2+} at 28.89 ± 2.48 %, NO• at 22.91 ± 3.5 % and •OH at 18.56 ± 0.11 %. The results of the present study demonstrates that celecoxib has potential of inhibiting nitric oxide, superoxide anion radical, hydroxyl radical, hydrogen peroxide as well as metal chelation *in vitro*.

Keywords Metal chelating, reactive oxygen, celecoxib, COX-2 inhibitor, *in vitro*

Introduction

Free radicals are thought to be involved in the development of a number of pathological conditions and diseases, especially of the cardiovascular system. The radicals involved in those processes are mainly oxygen-centered radicals such as superoxide, hydroxyl, or peroxyl radicals. The administration of antioxidative drugs or natural products to treat or prevent such conditions is of great interest [1].

Oxidants such as $O^{\bullet-}_2$, H_2O_2, $^{\bullet}OH$ and HOCl (hypochlorous acid) are formed at sites of inflammation, and appear to contribute to the tissue damage in some acute and chronic inflammatory diseases, such as rheumatoid arthritis, and some forms of adult respiratory distress syndrome [2]. It has therefore been suggested that many anti-inflammatory drugs might exert part of their action by scavenging oxidants [3].

CELEBREX (celecoxib) capsule is a nonsteroidal anti-inflammatory drug. The chemical name is 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene sulfonamide and is a diaryl-substituted pyrazole. The molecular weight is 381.38. Its molecular formula is $C_{17}H_{14}F_3N_3O_2S$. Celecoxib is a cyclooxygenase-2 (COX-2) specific inhibitor, a member of a larger class of non-Steroidal anti-inflammatory drugs (NSAIDs) that exhibits anti-inflammatory, analgesic, and antipyretic activities in animal models [4]. The mechanism of action of celecoxib is believed to be due to inhibition of prostaglandin synthesis, primarily by inhibition of COX-2. At therapeutic concentrations in humans celecoxib does not inhibit cyclooxygenase-1(COX-1). COX-2 is induced in response to inflammatory stimuli. This leads to the synthesis and accumulation of inflammatory prostanoids, in particular prostaglandin E2, causing inflammation, oedema and pain. In animal models, celecoxib acts as an anti-

inflammatory, analgesic and antipyretic agent by blocking the production of inflammatory prostanoids via COX-2 inhibition [5].

It has been extensively demonstrated that several non-steroidal anti-inflammatory drugs (NSAIDs) are effective scavengers of ROS and RNS, which probably contributes to their final therapeutic activity. The aim of the present study was to evaluate the scavenging activity of celecoxib against ROS using *in vitro* assays.

Materials and Methods

Materials

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene sulfonamide), sulfanilamide, pyrogallol, ammonium molybdate, 1, 10-phenanthroline, Sodium thiosulphate were obtained from Sigma-Aldrich (St Louis, MO, USA). All other chemicals used of analytical grade.

Superoxide Anion Scavenging Assay

Ability of Celecoxib to inhibit the autoxidation of pyrogallol was measured according to the modified method of Marklund and Marklund [6]: 0.3mL of Celecoxib (100 µM– 700 µM) and 2.61mL of 50mM phosphate buffer (pH 8.24 at 25 °C) was mixed in a cuvette. Freshly prepared 0.09mL of 3mM pyrogallol in 10mM HCl was added and the inhibition of pyrogallol autoxidation was measured at 325nm using UV-VIS spectrophotometer. Absorbance of each Celecoxib was recorded at every 1 min interval for 10 min and the increment of absorbance was calculated by the difference (the absorbance at 10 min – the absorbance at the starting time).

% Inhibition = (Change in Ac - Change in As) / Change in Ac x 100

Hydrogen Peroxide Scavenging Activity Assay

Hydrogen peroxide scavenging activity of Celecoxib was estimated by replacement titration according to the method of Zhang [7]. Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various Celecoxib (100 µM– 700 µM) were mixed, followed by 2 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM Sodium thiosulphate (NaS_2O_3) until yellow colour disappeared. Percentage of scavenging of hydrogen peroxide was calculated as: % Inhibition (%) = $(V_0\text{-}V_1)/V_0$x100; where V_0 was volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without Celecoxib), V_1 was the volume of NaS_2O_3 solution used in the presence of Celecoxib.

Fe^{2+} Chelation Assay

The Fe^{2+} chelating ability of Celecoxib was determined using a modified method of Minotti and Aust [8] with a slight modification by Puntel et al. [9]. Freshly prepared 500 µM $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl 0.1M Tris-HCl (pH 7.4), 218 µl saline and Celecoxib (100 µM– 700 µM). The reaction mixture was incubated for 5 min, before the addition of 13 µl 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the JENWAY UV-Visible spectrophotometer. The Fe (II) chelating ability was subsequently calculated as % Fe^{2+} chelation = $(ABS_{control} - ABS_{sample}) / ABS_{control}$x 100%.

$NO^{\bullet}$ scavenging activity

The scavenging effect of Celecoxib on NO• was measured according to the method of Marcocci and colleagues [9]. Briefly, sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) (pH 7.4) was mixed with different concentrations of Celecoxib (100 µM-700 µM) and incubated at 25 °C for 150 minutes. After incubation, nitrite produced from sodium nitroprusside was measured by Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% 1-naphthylethylenediamine dihydrochloride in water). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with 1-naphthylethyelenediamine dihydrochloride was immediately read at 570 nm. The percentage of NO scavenging was calculated using the following formula: (%) = (A0-A1)/A0x100 where A0 is the absorbance of the control and A1 is the absorbance of Celecoxib.

Hydroxyl Radical Scavenging

Hydroxyl radical scavenging activity was measured by the salicylic acid method [10] with some modifications. Briefly, Celecoxib was dissolved in double distilled water at (100 µM-700 µM). A 1.0 mL Celecoxib was mixed with 1.0 mL of 9 mmol/L salicylic acid, 1.0 mL of 9 mmol/L FeSO4 and 1.0 mL of 9 mmol/L H_2O_2. The reaction was activated by adding H_2O_2 and the reaction mixture was incubated for 60 min at 37 ^{o}C in a water bath. After incubation, the absorbance of the mixtures was measured at 510 nm using an ultraviolet/visible (UV/Vis) spectrophotometer. The percentage of hydroxyl radical scavenging was calculated using the following formula: (%) = (A0-A1)/A0x100 where A0 is the absorbance of the control and A1 is the absorbance of Celecoxib.

Statistics

Results

Table 1: Percentage metal chelating and reactive oxygen species scavenging of celecoxib

Celecoxib	•OH (%)	$NO^{\bullet}$ (%)	H_2O_2 (%)	$O^{\bullet-}_2$ (%)	Fe^{2+} (%)
100 µM	18.56 ± 0.11	22.91 ± 3.5	43.16 ± 3.12	33.23 ± 0.78	28.89 ± 2.48
300 µM	27.11 ± 1.23	38.54 ± 0.98	58.74 ± 2.31	47.64 ± 2.55	34.71 ± 3.67
500 µM	42.67 ± 3.21	44.86 ± 0.44	64.56 ± 3.51	59.97 ± 2.78	56.26 ± 0.66
700 µM	51.71 ± 1.90	67.99 ± 2.33	73.41 ± 1.77	68.49 ± 3.09	60.63 ± 1.79

Each experiment was repeated three times and values expressed as mean ± standard deviation.

Statistical analyses

Results are presented as means±standard deviation. The statistical evaluation of all data was done using SPSS.

Discussion

It has long been known that ROS are generated during inflammatory processes by means of the myeloperoxidase/H_2O_2/Cl^- system [12] through the reaction between H_2O_2 and HOCl. These ROS may also be formed in inflammatory processes as a byproduct resulting from the spontaneous dismutation of $O_2^{\bullet-}$. Once formed, in substantial amounts, ROS may play important role in mediating the destruction of infectious agents during host defense. On the other hand, it is common knowledge that a sustained production of ROS in prolonged or chronic inflammation can lead to severe damage to surrounding tissues, with consequences like cardiovascular disease, multiple sclerosis, diabetes, cancer, and dementia. Importantly [13], ROS are capable of damaging almost all biological molecules and are particularly genotoxic, which gives a special relevance to the scavenging of this ROS through anti-inflammatory treatments [14-15].

NSAIDs are used for the treatment of inflammatory processes, due to their ability to inhibit cyclooxygenase-2, a putative scavenging activity of NSAIDs for ROS would also be of high importance for their therapeutic effects.

H_2O_2 can be formed in vivo through the dismutation of superoxide anion and many oxidase enzymes. Hydrogen peroxide is not particularly reactive. Under physiological conditions, the reactions of H_2O_2 are mainly confined to its oxidizing ability. It can oxidize thiols and by so doing, inactivate enzymes that contain an essential thiol group. As hydrogen peroxide is fairly stable and can readily pass through membranes it can react with biological molecules far removed from its site of production [16]. A significant problem for living organisms is the consequence of the reaction between hydrogen peroxide and oxidizable metals, the Fenton reaction [17]. The present study showed a higher percentage scavenging of hydrogen peroxide in a dose dependent pattern. The highest percentage of inhibition of hydrogen peroxide was at 700 µM a. 73.41 ± 1.77 %. Although H_2O_2 is not a free radical but celecoxib inhibited the production of H_2O_2 these results are similar with the studies of Costa et al., [13] who also reported the scavenging effect of NSAIDs containing pyrazole derivatives against singlet oxygen.

Superoxide radical (O_2^-). is an oxygen-centered radical, this species is produced by a number of enzymess, by autooxidation reactions, and by nonenzymatic electron transfers that univalently reduce molecular oxygen. It can

also reduce certain iron complexes such as cytochrome c. Superoxide dismutase (SOD) accelerates the dismutation of $O_2^{\cdot-}$, converting it to H_2O_2 and O_2 [14-15]. This study indicated tha celecoxib neutralizes the effect of superoxide making it unavailable for the oxidation of pyrogallol also in a concentration dependent fashion. Our data also showed percentage scavenging of superoxide in a concentration dependent manner 33.23 ± 0.78 %, 47.64 ± 2.55 %, 59.97 ± 2.78 %, 68.49 ± 3.09 % respectively. These results are in consonant with the works of Toshiaki et al. [18], who also reported the scavenging effect of adrenergic agents against superoxide anion.

Chronic inflammatory processes cause a significant change in iron metabolism with a drop in serum iron and a redistribution of iron to the activated reticulo-endothelial system. Fe (iron) overload appears to selectively worsen joint inflammation whilst nutritional Fe deficiency has the converse effect. Inflammatory synovial fluid contains Fe in a form capable of generating the hydroxyl radical [19]. One feature of OH· is that it begets another radical, i.e., when it reacts with a molecule, the result is the formation of another radical species. The resulting species usually has lower reactivity than OH·. OH· attacks all proteins, DNA, PUFA in membranes, and almost any biological molecule it touches [14-15]. Many reports abound about the chelating capacities of NSAIDs utilizing the iron-EDTA system as described by Halliwell et al. [20]. Our present results are in line with the results of Karl et al. [21] who also reported that carvedilol chelated Fe^{2+} in the iron-EDTA system. Our data also showed percentage chelation of Fe^{2+} in a concentration dependent manner 28.89 ± 2.48 %, 34.71 ± 3.67 %, 56.26 ± 0.66 %, 60.63 ± 1.79% respectively.

NO• is produced from metabolism of the amino acid, L-arginine. The enzyme(s) catalyzing this metabolic pathway are known as nitric oxide synthases (NO• synthases, or NOS). NOS converts L-arginine to L-citrulline and NO• via a 5-electron oxidation of one of the guanidine nitrogens of L-arginine [22]. NO•, a free radical gas, is an important intracellular messenger molecule that activates the soluble guanylyl cyclase (sGC) at nanomolar concentrations. Guanylyl cyclase is responsible for converting guanosine triphosphate (GTP) to cyclic GMP, which causes smooth muscle relaxation [23]. Nitric oxide (NO•) is an important mediator of diverse physiologic and pathologic processes, including arthritis. High levels of NO production results in direct tissue toxicity and contributes to various carcinomas and inflammatory conditions [24]. Our present experimental data also showed percentage scavenging in a concentration dependent manner 22.91 ± 3.5 %, 38.54 ± 0.98 %, 44.86 ± 0.44 %, 67.99 ± 2.33 % respectively. The reaction between NO•· and $O_2^{\cdot-}$ forms peroxynitrite (ONOO−), which have •OH like potential leading to cytotoxicity.

The present results showed that celecoxib at concentration of 100-700 µM scavenged the notorious hydroxyl radical based on the method described by Smirnoff and Cumbes [11]. The results are showed in table 1, which are similar to the reports of Toshiaki et al. [18] and Karl et al. [21]. The hydroxyl free radical (HO•) is the most reactive ROS formed *in vivo*. The hydroxyl free radical can be formed by a number of processes including the Fenton reaction, the Haber-Weiss reaction, and the homolytic fission of water molecules (e.g., by ionizing radiation). It can also be produced by the decomposition of ozone under aqueous conditions [25].

Conclusion

The results of the present study demonstrated that celecoxib has potential of inhibiting nitric oxide, superoxide anion radical, hydroxyl radical, hydrogen peroxide as well as metal chelation in vitro. The results of celecoxib at 100 µM scavenged H_2O_2 at 43.16 ± 3.12 %, $O_2^{\bullet-}$ at 33.23 ± 0.78 %, Fe^{2+} at 28.89 ± 2.48 %, NO• at 22.91 ± 3.5 % and •OH at 18.56 ± 0.11 %.

References

[1]. Halliwell, B., and Gutteridge, J.M. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J; 219:1–14.

[2]. Halliwell, B., and Gutteridge. J.M.C., (1985). The importance of free radicals and catalytic metal ions in human diseases. *Molecular Aspects* of *Medicine,* 8, 89-193.

[3]. Wasil M., Halllwel, L.B, Moorhouse, C.P., Hutchison. D.C,S., and Baum, H., (1987). Biologically significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by some anti-inflammatory drugs. Biochemical Pharmacology, *36*, 384773850.

[4]. Hilmi, I., and Goh, K.L. (2006). Chemoprevention of colorectal cancer with nonsteroidal anti-inflammatory drugs. Chin J Dig Dis 7(1):1–6.

[5]. Fort, J. (1999). Celecoxib, a COX-2-specific inhibitor: The clinical data. Am J Orthop (Belle Mead NJ) 28(3):13–18.

[6]. Marklund, S., and Marklund, G. (1974).Involvement of the superoxide anion radical in the autoxidation of pyrogallol and convenient assay for superoxide dismutase. European Journal of Biochemistry 47: 469–474.

[7]. Zhang, X.Y. (2000) Principles of Chemical Analysis. Beijing: China Science Press;. 275.

[8]. Minotti, G., and Aust, S.D. (1987). An investigation into the mechanism of citrate-Fe^{2+}-dependent lipid peroxidation. Free RadicBiol Med; 3: 379–387.

[9]. Puntel, R.L., Nogueira, C.W., Rocha, J.B.T. (2005). Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in rat brain *in vitro*. Neurochem Res; 30:225–35.

[10]. Marcocci, I., Marguire, J.J., Droy-lefaiz, M.T., Packer, L., (1994). The nitric oxide scavenging properties of Ginkgo biloba extract. Biochem. Biophys. Res. Commun. 201, 748–755.

[11]. Smirnoff, N., Cumbes, Q.J. (1989). Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry, 28(4): 1057-1060.

[12]. Schiller, J., Fuchs, B., Arnhold, J., and Arnold, K. (2003). Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: molecular pathways, diagnosis and potential therapeutic strategies. *Curr Med Chem*; 10: 2123–2145.

[13]. Costa, D., Gomes, A., Lima, J.L.F.C., and Fernandes, E., (2008). Singlet oxygen scavenging activity of nonsteroidal anti-inflammatory drugs Redox Report Vol 13 No 4 153-160.

[14]. Aruoma, O I. (1998). Free Radicals, Oxidative Stress, and Antioxidants in Human Health and Disease *Journal of Amercan Oil Chemst;s Socety 75*, 199–212

[15]. Aruoma, O I. (1996). characterization of drugs as antioxidant prophylactics. Free Radical Biology & Medicine, Vol. 20, No. 5, pp. 675-705

[16]. Makino, N., Mochizuki, Y., Bannai, S., and Sugita, Y. (1994). Kinetic studies on the removal of extracellular hydrogen peroxide by cultured fibroblasts. J. Biol. Chem., 269, 1020-1025.

[17]. Koppenol, W.H. (1993). The centennial of the Fenton reaction. Free Radical Biology and Medicine 15:645-651.

[18]. Toshiaki M., Sanae M., and Taketo O.(1998). Antioxidant Activity of Adrenergic Agents Derived from Catechol Biochemical Pharmacology, Vol. 55, pp. 2001–2006.

[19]. Blake, D.R., Gallagher, P.J., Potter, A.R., Bell, M.J., and Bacon, P.A. (1984). The effect of synovial iron on the progression of rheumatoid disease. Arthritis and Rheumatism; 27: 495-501.

[20]. Halliwell. B., Gutteridge, J.M.C., and Aruoma O.I., (1987). The deoxyribose method: asimple 'test tube' assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, 165, 215-219.

[21]. Karl O., Joachim G., Klaus Z., Ernst H., Gilbert R., and Gunther J. (2001). Radical-scavenging and iron-chelating properties of carvedilol, an antihypertensive drug with antioxidative activity. Biochemical Pharmacology 62:241–248.

[22]. Murad, F. (2006). Nitric oxide and cyclic GMP in cell signaling and drug development. New England Journal of Medicine 355:2003–2011.

[23]. White, K. A.; Marletta, M. A. (1992). Nitric oxide synthase is a cytochrome P-450 type hemoprotein. Biochemistry 28:6627–6631.

[24]. Abramson, S.B., Amin, A.R., Clancy, R.M., and Attur, M. (2001). The role of nitric oxide in tissue destruction. Best Pract Res Clin Rheumatol; 15:831-845.

[25]. Hoigne, J., and Bader, H. (1975). Ozonation of water: Role of hydroxyl radicals as oxidizing intermediates. Science, 190-792.