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Therapeutic applications of nitric oxide releasing non steroidal anti- inflammatory drugs

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Abstract: This review examines the therapeutic potentials and mechanism of action of nitric oxide releasing non-steroidal anti-inflammatory drugs. NO-releasing NSAIDS markedly improve the anti-inflammatory and antinociceptive actions efficiently and diminish gastrointestinal toxicity. The nitric oxide releasing NSAIDS may be useful to treat wide variety of diseases like colitis, cancer, thrombosis, restenosis and bronchial asthma.

Key words: NO-NSAID, gastrointestinal disease, inflammation, cancer.

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Introduction

Nitric oxide (NO) – releasing non steroidal anti inflammatory drug (NSAIDS) is a novel class of compounds. These compounds have limited significant side effects. In vitro and vivo studies indicate that they are much more effective than parent NSAIDS in modulation of colonocyte kinetics[1]. NSAIDS induced upper gastrointestinal disease remains a major problem that affects a broad segment of the population, by frequent prescription and over the counter dispensing. NO–releasing aspirin and COX-inhibiting NO-donors are potential alternatives to traditional NSAIDS with upper gastrointestinal toxicity [2].

Estimated 3-4 million Americans take NSAIDS daily. This new class of NO-NSAIDS is prepared by adding a radical, nitro butyl or nitrosothiol by using a short chain ester linkage. This exhibits reduced gastrointestinal toxicity while enhancing vasodilatation, reducing blood platelet adhesion and acting as a buffer against memory loss[3].They are synthesized by ester linkage of an NO- releasing moiety to conventional NSAIDS, such as aspirin (NO-Aspirin), flurbiprofen (NO-flurbiprofen), naproxen (NO-naproxen), diclofenac (Nitrofenac), lbuprofen(NO-lbuprofen) and indomethacin (NO-indomethacin).

The NO-NSAIDS overcome the gastric injury caused by NSAID. The capacity of NO-NSAID, to release NO appears to reduce the gastrointestinal toxicity[4]. The various mechanisms that underline the protective effect of NO in the stomach includes vasodilatation of the mucosal blood vessels, inhibition of caspase enzymes activity and inhibition of leukocyte adhesion

NO NSAIDS and the gastrointestinal tract

Gastrointestinal damage is an important side effect of NSAID which has been attributed to inhibition of gastric COX-l activity leading to loss of prostaglandin (PGI_2, PGE_2) formation.An experimental study with NO-NSAID showed their ability to spare the gastrointestinal tract after either acute or chronic use in animals, NO-naproxen is completely devoid of ulcerogenic activity [5]. Similar results have been reported with NO-Aspirin after single dose administration in rats [6,7] nitrofenac [8,] NO-indomethacin [4], and NO-flurbiprofen failed to damage the rat small intestine, while hemorrhagic lesions were observed in animals treated with flurbiprofen [8,9]. The administration of NO-NSAID in acute conditions of diseases is associated with markedly less toxicity   than the parent NSAID both the intestine and stomach.

NO-Flurbiprofen significantly restored gastric blood flow which was reduced by bacterial lipopolysaccharrides (LPS) administration. In LPS-induced of gastric damage in animals [10]NO-aspirin reduced the fall in gastric blood flow throughout the shock period [11]NO-aspirin had no proulcerogenic activity [12] NO-aspirin accelerated the healing process. NO-aspirin showed a dose dependent decrease in the severity of HCl/ethanol induced stomach lesions in rats [13]No-NSAID may be valuable in the treatment of existing ulcers and are likely to be of greater therapeutic benefit than classical NSAID for the treatment of inflammatory disease in patients with pre existing gastric damage.

NO-Aspirin and NO-Naproxen and their native aspirin and Naproxen failed to affect expression of cyclooxygenase-1 mRNA, but unregulated the cyclooxygenase-2 mRNA.[14]   NO-indomethacin partially suppressed membrane permeability and the inhibitor for Guanylate cyclase suppressed the cyto protective effect of NO-inodomethacin against celecoxib.[15] COX inhibiting NO-donating drug (CINOD) inhibits COX-1 and COX-2 activities, has less adverse effect on gastrointestinal tract and reduce systemic blood pressure. NO-ASA (Acetyl Salicylic Acid)maintains gastric mucosal blood flow and reduces leukocyte – endothelial cell adherence[16].

NO-ASA counteracts the impairment of ulcer healing in diabetic rats induced by ASA, mainly due to the release of NO that compensates prostaglandin deficiency resulting in enhancement in the Gastric blood flow at ulcer margin and suppresses the cytokine release in the ulcer area[17]. NO-aspirin does not undergo biotransformation in the upper gastrointestinal tract and the stomach acts as a reservoir for the dug.[18] Aceylation of cycloxygenase (COX-2) by aspirin can trigger the formation of aspirin triggered lipoxin (ATL). ATL exerts protective effects in the stomach. NO–aspirin may be an alternative to aspirin for indication such as cardio production and COX-2 inhibitors [19].

NO–ASA exhibits mucosal protective and healing effects against WRS-induced gastric lesions due to the release of NO which induces gastric hyperemia, attenuation of lipoid peroxidation and counteracts the inhibition of HSP 70 expressions induced by native ASA [20]. NO-indomethocin probably by releasing NO, exerts protective influence, such as increase of gastric mucosal blood flow that counteract, the potential damaging effects of cyclooxygenase inhibition by indomethacin [21]. The gastric sparing effect of NO-Aspirin is due to an increase of gastric mucosal blood flow meditated by releasing from this drug [22] . NO-ASA failed to affect healing of gastric ulcers and failed to produce the rise in the plasma **1l-1b** levels and increased lipid per oxidation as compared to those recorded in ASA treated animals [23].

NO-releasing derivatives failed to affect expression of COX-1 mRNA but unregulated the cyclooxygenase 2 mRNA (concurrent inhibition of COX- 2) by selective inhibitors which by it delayed ulcer healing and attenuated the gastric blood flow at ulcer margin). NO–NSAID unlike classic non-steroidal anti inflammatory drugs does not affect gastric mucosa and fails to delay the healing of pre existing ulcers [24].

NO Aspirin spares the gastric mucosa and inhibits Caspase activity through cGMP-dependent and independent pathways and caused caspase inactivation by s-nitrosylation (inhibition of tumor necrosis factor (TNF) alpha release or activity by TAPL-2 or anti TNF- alpha reception monoclonal antibodies protected against mucosal damage and caspase activation). NO–Aspirin protected gastric chief cells from toxicity induced by TNF-alpha by activating cGMP dependent pathways [25]. Increasing the endogenous biosynthesis of NO selectively at gastric mucosa level [26] .NO-Aspirin caused a marked increased in mucosal blood flow with no effect on potential difference and PH, NO-Aspirin neither had a topical irritating action on the stomach nor exerted a worsening effect on gastric ulcerogenic to stress [27] NO-NSAIDs (Nitrofinac) are capable of accelerating tissue repair [28].

Vasodilatation of the gastric vasculature

The prostaglandins normally protect the mucosal lining against injurious stimuli by various mechanisms one of which is dilation of mucosal blood vessels. In the presence of NSAID, vasodilator PGI_2 and PGE_2 production are diminished leading to constriction of mucosal blood vessels with a potential for ischemia, leukocyte entrapment and sub-sequently leading to stomach ulceration and hemorrhage.   In rats, flurbiprofen constricted post capillary venues by 16.6% while No-flurbiprofen dilated these vessels by 6.7% 1. In diclofenac administered rats, the was a gradual reduction in gastric blood flow to about 50%, while nitrofenac did not affect blood flow [29] A vasodilatory effect of NO-released from, NO-NSAID administration most probably plays a significant part in minimizing the ulcerogenic potentials of these compounds.

Inhibition of leukocyte adhesion

The NSAID induced gastric damage, was found to be a process [30,31] where flurbiprofen significantly increased leukocyte adherence to mesenteric post capillary venules in rats [4]. The NO-NSAIDs have been shown to reduce transduction via the NF-KB pathway which is an important route to expression of leukocyte adhesion molecules [30] and thus the leukocyte sticking to endothelium cannot be discounted.The NO released from NO NSAID, like PGI_2, also inhibits

Neutrophil adhesion to the blood vessel wall. The NO replaces the lost PGI_2 not only as a mucosal vasodilator but also as an endogenous inhibitor of Neutrophil activation [32].

Caspase Inactivation

Caspases are a family of cysteine proteases that resemble interleukin-1 β (1L-1 β) converting enzyme (ICE). The caspase primarily is involved in cytokine release, clearing proIL-1 β to produce lL-1β [33] and to lesser extent, lL-8 interferon γ (IFN-γ) [34]. The activation of caspase-3 like enzyme is the major pathway involved in cytokine induced apoptosis [35]. In aspirin pretreated rats, increased gastric mucosal activity of both caspase [4-7] and NO aspirin caused post translational inactivation of caspase in direct manner by S-nitrosulating cysteine residues in the enzyme core [7,36] .The exposure of gastric mucosal cells to flurbiprofen resulted in concentration dependent apoptosis while No flurbiprofen inhibited apoptosis and caspase activity in those cells [37]. NO aspirin has been shown to protect the gastric cells from TNF and induced toxicity by activating CGMP dependent pathways that leads to inactivation of caspase-3. The inactivation of caspase appears to be an important factor in the gastrointestinal tolerability of NO NSAID.

NO-flurbiprofen delayed disease onset and significantly decreased disease severity and it was associated to i. decreased MRNA levels of proinflmmatory cytokines caspase-1, and iNOS in blood cells. ii. decreased ability of encephalitogenic T-cells to proliferate. iii. Reduced number of central nervous system infiltrating T cells. iv. decreased axonal loss and demyelination. and V.increased CD4+ CD69-CD25+ regulatory T cell in the spleen[7].NO-NSAIDs are potent inhibitors of T helper (Th1) type cytokines and the effect owing to post translational nitrosation and inactivation of cysteine proteases, interleukin (1L) -1βconvertingenzyme(ICE/Caspase-1)involved inprocytokine processing[39]molecular mechanism underlying this action of nitroacetaminophen is not clear. Evidence for inhibition of cytokine directed formation of pro-inflammatory molecule production. (eg.COX-2, iNOS) by an effect on the NF-kappa β transduction system or nitrosylation of caspase enzyme activity has been reported[40].

NO NSAIDS and inflammation

Antioedema and antiarthritis

NO-paracetamol and paracetamol exhibit similar antipyretic activity in rats challenged with LPS [41]. NO-paracetamol was significantly more potent than paracetamol as an inhibitor of carrageenan induced rat hind paw oedema [42] Nitrofenac showed similar effect to that of diclofenac in carrageenan induced rat hind paw oedema [43] NO-naproxen and naproxen [5]and NO-indomethacin and Indomethacin [6]also showed similar anti oedema effects in this anti-inflammatory models chronic treatment with NO-naproxen or naproxen [44]and nitrofenac or diclofenac [45] elicited similar anti-inflammatory activity in freund's adjuvant models of arthritis in the rats. NO-prednisolone exhibited potent anti-inflammatory activity in variety of models of inflammation including zymosan induced peritonitis and granulamatous inflammation [46]. NO-prednisolone proved to be more potent than prednisolone on a molar basis. ASA and Nimesulide do not influence NO concentration; But Diclofenac causes an increase in NO blood concentration in rats[47].The balanced inhibition of the two main COX isoforms with release of NO confers to NO–NSAIDs reduced gastrointestinal and Cardio renal toxicity. NO which

released as the compounds are broken down, may counter act the consequences of the NSAIDS induced decrease in gastric mucosal prostaglandin[48] . Up regulation of COX-2 expressions has been implicated in the pathophysiology of Neuronal cell death. NO-induced up regulation of COX-2 via activation of activator protein-1 (AP-1) signaling leads to apoptotic cell death. NO induced upregulation of COX-2 via activation of activator protein-1 (AP-1) signaling leads to apoptotic cell death. These results suggested that excessive NO production during inflammation induces apoptosis in pheochromocytoma cell through AP-1 mediated upregulation of COX-2 expression [49].

Brain inflammation is an underlying factor in the pathogenesis of Alzheimer's disease. NO-donors potentiate the anti-inflammatory activity of fluribrofen in models of brain inflammation [50]. NO-Aspirin delivered to PMBC derived T lymphocytes and monocytes causes a transitory inhibition of cell respiration and reduction of cellular ATP, which translates in time reversible inhibition of cell proliferation and 1L-2, 1L-4, 1L-5 and IFN-gamma secretion. NO-Aspirin enhances glucose uptake, glycolytic rate and lactate generation in CD3/CD28 co stimulated lymphocytes, reduced citric acid cycle intermediates. No Aspirin causes a metabolic hypoxia that inhibits lymphocyte reactivity to costimulatory molecules, providing a potential counter mechanism to control activated immune system[51].

NO-NSAIDs have been reported to have additional anti-inflammatory and immuno modulatory property compared to parent compounds. Treatment with flurbiprofen was associated with decrease in mRNA levels of pro-inflammatory cytokines, caspase-1, and iNOS in blood cells, decreased ability of encephalitogenic T cells to proliferate, reduced number of central nervous system (CNS) infiltrating T-cells, decreased axonal loss and demyelination, increased CD4(+) CD69(-) CD25(+) regulatory T cells in the spleen [52].

NO-releasing flurbiprofen has been shown to be effective in reducing beta- amyloid deposition in transgenic mouse models of Alzheimer's disease. NO-flurbiprofen demonstrated that a single dose administration can produce prolonged suppression of brain prostaglandin synthesis without causing gastric injury[53].

NO-releasing mesalamine suppresses inflammatory cytokine production and reduces leukocyte infiltration[54].NO-ASA prevents monocyte tissue factor expression. This is accompanied by inhibition of TX and cytokine biosynthesis[55].Nitroparacetamol exhibit hepatoprotection. NO-NSAIDs of disease states including pain and inflammation, thrombosis and restenosis neurodegenerative disease of the central nervous system colitis, cancer, urinary incontinence, liver disease, impotence, bronchial asthma and osteoporosis [56] NO-ASA treated mice was associated with a marked reduction in CD45-positive inflammatory cells and an increased number of tunel-positive cells. Released NO, can reduce vascular inflammation and promote apoptosis during vascular remodeling associated with neointimal thickening [57].

NO-NSAIDs is more efficacious than ibuprofen or celecoxib in clearing a beta deposits from than brains of Tg Mice, implying potential benefit in the treatment of Alzheimer's dementia[58]. NO-NSAIDS may play an important role among the long term treatment of chronic inflammatory oseoarticular and rheumatic diseases[59].NO-aspirin causes intracellular NO formation and suppresses 1L-1beta and 1L-18 processing by inhibiting caspase-11-activity

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caspase-1 inhibition is a new Cyclooxygenase-independed anti-inflammatory mechanism of NO-aspirin [60]. NO-naproxen inhibited T-cell proliferation and reduced both 1L-1beta and TNF alpha plasma levels. Introduction of the NO moiety in the naproxen structure increases the effect at the levels of the immune system[61].

As NO-Naproxen significantly enhances collagen deposition at a wound site, it can be used as anti-inflammatory and analgesic agents in post surgery patients[62]. NO–releasing derivative of mesalamine has significantly enhanced anti-inflammatory activity, improved efficacy in a rat model of colitis. This efficacy is most likely caused by it enhanced ability to suppress leukocyte infiltration and possibly to scavenge peroxynitrite[63].

Irritable bowel disease and colitis

Mesalamine is one of the most commonly used drugs for treatment of inflammatory bowel disorder. NO-mesalamine was significantly more effective than mesalamine No-mesalamine and mesalamine inhibited N-formyl methionyl-leucyl- phenylalanine (FMLP) induced leukocyte adherences to mesenteric vascular endothelium. NO-mesalamine significantly reduced vascular colonic tissue levels of Myeloperoxidase (MPO) .The vasodilatation by NO may have contributed the reduction of colonic damage [64].

The ability of NO NSAID to inhibit caspase (ICE) activity reduces the formation of pro inflammatory lL-1β[7]. NO-prednisolone also reduces lL-1 β formation by LPS treated human peripheral blood mononuclear cells [46]. No aspirin dose dependently inhibits IL-6 and tumor necrosis factor (TNF-γ) formation by LPS challenged human monocytes [65].The nitrofenac and diclofenac were equipotent as inhibitors of Cox-l and Cox-2 [66]. NO aspirin and NO paracetamol inhibit induction of COX-2 and induce nitric oxide synthase (iNOS) in LPS pretreated cultured J774 macrophage [67]NO NSAID reduced iNOS in inflammatory cells in LPS challenged brain microglial culture NO flurbiprofen actually increases expression of these enzyme [68].

NO NSAID and Pain

The main therapy application of NSAID is analgesia. The PGE_2 and PGI_2, synthesized at the site of inflammation recognized to sensitize the firing of peripheral nociceptors. The NSAID induced inhibition of prostaglandin production accounts for the analgesia activity of these compounds in inflammatory pain and hyperalgesia. NO-donors drugs, acting in the spinal card, might perhaps, are expected to promote pain perception. NO-donors acting at peripheral site have been demonstrated to reduce pain perception. In chronic treatment with NO-naproxen resulted in greater anti nociecption in freund's adjuvant induced rat model of arthritis [44]. NO-aspirin [69]and NO-paracetamol [42] are more effective in the mouse acetic acid induced abdominal constriction test than the parent compound and also produced greatest anti-nociception in rats in which hyperalgesia induced by carrageenan [42,69]. The enhanced anti nociceptive activity of NO-NSAID is not clear.

Cardio Vascular Disease

NO-Naproxen by donating NO displays a noticeable anti-ischaemic effect. In reperfused ischemic rabbit hearts, its ability controls experimental hypertension, suggest that may have therapeutically potential in cardiovascular diseases and prevention the myocardial ischemic events[70].

NO-aspirin prodrug constitutes a potentially beneficial property for the prophylactic prevention of thrombus formation and adverse cardiovascular events such as stroke and myocardial infarction[71].NO-releasing ASA derivative stimulates reparative angiogenesis, also   prevents apoptosis and oxidative stress, there by alleviating the consequences of supervening arterial occlusion[72]. NO–aspirin treated rats .The cGMP increased significantly .Increased cGMP in vascular tissues, reduces sympathetic mediated vasoconstriction in resistance vessels and lowers blood pressure in spontaneously hypertensive rats[73].

NO-Aspirin reduced significantly plasma LDL oxidation. Chronic treatment with NO-containing aspirin has antiatherosclerotic and antioxidants effects in the arterial wall of hypercholesterolemic mice[74].NO–aspirin improved protection for the heart[75].NO-releasing devices as well as innovative improvements to conventional NO donors. Several examples are given in some important therapeutic indications such as cardiovascular diseases (NO-Aspirin). Pain and inflammation (NO-paracetamol), osteoporosis and urinary incontinence (NO-flurbiprofen with aliphatic space respiratory disorders (NO-steroids)[76].

NO-donors releasing nitric oxide increase myocardial production of prostanoids[77]. NO-aspirin appears to exert a relevant cardio protection likely mediated by nitro oxide donation. These results suggest that this nitrodervative of Aspirin may leads to innovative therapy in myocardial ischemia and infarction[78]. ASA reduced PGE_2 synthesis and had slight inhibitory action also on NO production 1L-1 induced. The synthesis of PGE_2 and NO in articular cartilage[79].NO-Aspirin did not alter systemic arterial blood pressure when administered intravenously to the rat[80].

NO-aspirin, NO-prednisolone, NO-flurbiprofen and NO-paracetamol, released the noradrenalin. In precontracted rat aorta and NO-flurbiprofen is additionally, vasodilator in the perfused rat renal vascular bed .NO-NSAID are considerably less potent vasorelaxants than classical nitro vasodilators [81].The lack of vasorelaxant effect of NO NSAID *in Vitro* is reflected in the inability of these compounds, to reduce systemic blood pressure in normotensive animals *in vivo*. Thus NO-NSAID produced no significant change in blood pressure or heart over the following several hour [4]. NO-naproxen significantly reduced blood pressure in models of hypertension induced by occluding one renal artery [82]. NO-flurbiprofen has no effect on blood pressure of anesthetized rats [83].

NO-aspirin did not affect blood pressure in the anesthetized pig. The long-term administration of NO-naproxen increased blood pressure in conscious rats [79].The mechanism underlying the lack of vasopressor activity of NO-NSAID in normotensive animals is not clear. The ability of NO-NSAID to lower blood pressure in hypertensive animals cannot be explained. Further experiments to examine this possibility would be of value.

Platelets and Thrombosis

The aspirin is used for the prevention and treatment of cardio vascular disease. Aspirin exerts its antithrombotic effect by irreversible inhibition of COX-1, thereby reducing formation of proaggregatory TXA2 by platelets and antiaggreagetory, PGI_2 by vascular endothelial cells [84]. NO-aspirin can be expected to exhibit a greater degree of inhibition of platelet function. The increased antithrombotic potency of NO-aspirin relative to aspirin has been observed both *in vitro* and *in vivo*. Thus NO-aspirin inhibited platelet aggregation in rat and human platelet *in vitro* with greater potency than aspirin [85,86].

NO-aspirin was found more effective than aspirin in reducing the collagen or adrenaline induced fall in circulating platelets. NO-aspirin was effective against mechanically induced thrombus formation and supports the possibility that vasodilatation by NO-aspirin may contribute to its protective effect in this models[87].Collagen induced thromboenbolism in mouse, can also be reduced by pro treatment with flurbiprofen, flurbiprofen is also effective but significantly less potent than NO- flurbiprofen [88].

NO-releasing moiety in aspirin inhibits platelet aggregation by a combination of NO generation and COX inhibition[89]. NO-aspirin has shown improved antiaggregatory activity and also has been shown to exert protective effects in the gastrointestinal tract exposed to other injurious agents. NO-aspirin significantly inhibit leukocyte adherence, to the vascular endothelium which contribute the anti-thrombotic activity[90].NO-aspirin inhibited AA –induced platelet aggregation as well as serum TXB (2) generation induced by AA[91]. ASA reduced PGE_2 synthesis and had slight inhibitory action. The synthesis of PGE2 and NO in articular cardilage[79].NO-aspirin did not alter systemic arterial blood pressure when administered intravenously to the rat. Nitrobutylester derivative of aspirin did not inhibit platelet TX synthesis or gastric prostaglandin synthesis. Nitrobutylester derivative of Aspirin released nitro oxide when incubated in the presence of platelets and increased platelet levels of cGMP[80].

Atherosclerosis & Restenosis

NO-aspirin has been found to reduce restenosis to a greater degree than aspirin in a model pf restenosis in hyper cholestrolaemic mice [92]. The beneficial effect of NO flurbiprofen in a rat of vascular injury and restenosis has also been observed No flurbiprofen significantly reduced neointimal proliferation following percutaneous coronary angioplasty (PTCA)[93]. NO-aspirin inhibit growth of cultured rat aortic smooth muscles cells, by S-nitrosylation of ornithin decorboxylase [94].

NO-aspirin reduced ischaemia induced damage to the isolated perfused rabbit heart, while aspirin exacerbated the resulting myocardial   dysfunction[95]. NO-aspirin reduced myocardial injury following ischaemic and reperfusion in the pig [96].

Cancer chemotherapy

In the formation of premalignant colon lesions, NO-NSAIDS may play a highly promising role in chemoprevention of colon cancer [97].NO-ASA inhibits the growth of colon cancer cells, NO-

NSAIDS inhibits the growth of cultured cancer cells with greater potency than traditional NSAIDS. NO-ASA inhibits the growth of variety of human cancer lines, like pancreatic, colon, prostate, lung, tongue, breast and hematological tumors [98].

NO-NSAID may be useful in the management of bladder and prostate cancer [99]. All–NO-NSAIDS (ASA, salicyclic acid, Indomethacin, Sulindac, ibuprofen, flurbiprofen, piroxicam) have greater potency in inhibiting HT-29 and HCT-15 colon cancer cell growth compared to their parent NSAIDS. The inhibitory effect is due to a profound cell kinetic effect consisting of reduced cell proliferation and enhanced cell death. NO-NSAID has greater potency in inhibiting colon cancer cell growth[100].

The novel NSAIDS may display greater safety and greater efficiency compared to their parent NSAID and promise as chemo preventive agents against human colon cancer[101].

The degree of inhibition was pronounced with NO-Indomethacin, NO-aspirin at both dose levels than with NO-indomethacin at 80ppm and NO-aspirin at 3000 ppm significantly inhibited the colon tumors, total cyclooxygenase (COX) including COX-2 activity, Formation of prostaglandin E2 (PGE2), PGF2 alpha, 6-keto PGF1 alpha, and TxB2 from arachidonic acid. Nitric oxide synthase 2 (NOS-2) activities and beta catenin expression were suppressed in NO-NSAIDs given animals. This study provides strong evidence that NO-NSAIDs possess strong inhibitory effect against colon carcinogenesis. The effect is associated with the suppression of COX and NOS-2 Activities and beta – catenin levels in colon tumors [102].

NO-donating aspirin derivative on three human pancreatic adenocarcinoma cell lines: The cytotoxic action of this drug may due to hyperexpression of Bax, its translocation of the Mitochondria, the release of cytochrome C, activation of caspases-9 and -3, overall in a P53 independent manner. The experimental models showed that cox-2 hyper expression could partially explain the resistance mechanism to NO-ASA derivative [103].

NO-ASA reduced the incidence and multiplicity of pancreatic cancer (in Hamster models) NO-ASA arrested the transition from pan IN2 to panIN3 and carcinoma, NO-ASA suppressed it significantly during all stages except Pan IN1a. P21 (WAF1/CIPI), undetectable in normal cells, was progressively induced in neoplastic cells and suppressed by NO-ASA up to panIN3, Nuclear factor-kappa13 activation, absent in normal tissues, increased progressively (17 Fold in Cancer) suppressed by NO-SA in panIN3 and Carcinoma. Thus NO-ASA profoundly prevented pancreatic cancer and modulated multiple molecular targets in this model system and conventional ASA had no such effect [104].

NO-ASA formed a conjugate with glutathione, depleting glutathione stores. NO-ASA inhibited Wnt signaling by a dual mechanism, at low concentration it blocked the formation of beta-catenin / Tcf complex (dominant mechanism) at higher concentrations it also cleaved beta – catenin. These findings provides a mechanism of action by a potent chemopreventive, agent, underscore the significance of these pathways in regulating cell death in the context of cancer chemoprevention [105].

NO-ASA on drug metabolizing enzymes in HT-29 human colon adenocarcinoma and Hepa kk7mouse liver adenocarcinoma cells and in Min mice treated with NO-ASA, NO-ASA induced the activity and expression of (NAD(P)H:quinine oxireductases (NQO) and glutathione S-transferase (GST). NO ASA increased in the liver the activity of N_2O and also in intestine, the expression of NQO2, GST and P1-1 was also increased. NO-ASA induces phase ll enzymes, through the action of NO that if release and by modulation the keep1-NrF2 pathway this effect may be the part of. Mechanism of action against the colon and other cancers [106]. To investigate the cell death inducing mechanism of NO-NSAIDS, they analyzed gp-170, caspase expression and mitochondrial membrane potential depolarization or delta Psi depolarization. NO-NSAID showed striking cytocidal activity in both bladder cell lines (HT1376 and MCR). Apoptosis was triggered and is associated with active caspase-3 expression and delta Psi depolarization. In both cell lines, NO-NSAID causes apoptosis via mitochondrial – dependent mechanism could prove to be a useful agent in bladder cancer treatment [107]. The NO-ASA induced changes in PPAR delta expression correlated significantly with changes in apoptosis, NO-ASA suppresses intestinal tumorigenesis possibly in part through its inhibitory effect on PPAR delta expression [108]. NO-ASA inhibits both the expression and enzymatic activity of NOS 2 and these effects may represent an important mechanism for the colon cancer chemo preventive effect NO-ASA also decreased the corresponding steady state mRNA levels [109] NO-ASA inhibits the growth of colon, pancreatic, prostate, lungs, skin, leukemia, and breast cancer and up to 6000 folds more potent than traditional ASA (inhibition of NF-Kappa B activation. MAPK (Mitogen-activated protein kinase) signaling[110].

NO-NSAID has greater potency in inhibiting colon caner cells. The growth inhibitory effect is due to a profound cell kinetic effect consisting of reduced cell proliferation and enhanced cell death [111]. NO-NSAID, additional therapeutic applications in cardiovascular disease, Alzheimer's diseases and cancer. The increase in endogenous NO via a selective increase in inducible NO synthase in the gastric tolerability and the gastro protective effects [112].Preincubation with nitroparacetamol or nitroflurbiprofen cause of dose related inhibition of the formation of interleukin 1 beta and tumour necrosis factor alpha[113]. The metabolism of NO-ASA by these cells is characterized rapid deacetylation step and the formation of a conjugate with glutathione[114].

NO-Aspirin has demonstrable innovative properties for treatment of vascular disorder and cancer. NO-flurbiprofen has shown encouraging results in models of Alzheimer's diseases [115]. NO-NSAIDs may play a highly promising role in the chemoprevention of colon cancer [116,117]. NO-NSAIDs blocked the h(o)-h(1) to S cell cycle transition. This makes them promising candidates for chemopreventive agents against colon cancer[118].

Bone, Calcium and Osteoporosis

Prostaglandin has a regulatory effect on bone resorption. The principle effect of PGE_2 is to stimulate both bone resorption and formulation [119].Flurbiprofen depresses bone resorption in young rats without lowering bone formation and decrease in begin [120,121]. NO-flurbiprofen treated mice showed little change in Bone mass index (BMD), *in vitro* NO-flurbiprofen strongly inhibited both bases & lL-1-stimulated osteoclast formation and resorption while flurbiprofen had little effect on osteoclast and reversed the effect of lL-1 [122].

Conclusion

NO-NSAID are novel compounds with a different pharmacological activity to that of respective parent molecules NO-NSAIDs exhibit distinct   advantages over these respective parent compounds. These compounds are likely to be useful tool with which to prove further the biological roles of NO within the body. Furthermore animal and human studies are required to unravel the complexities of the mechanism of action and uses.

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