HIGHLIGHTS

NEW ANTI-INFLAMMATORY AGENTS: NO-NSAIDs AND COX-2 INHIBITORS
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Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to relieve pain and symptoms of arthritis and soft tissue inflammation. Most patients with rheumatoid arthritis receive NSAIDs as first-line agents for prolonged periods; however, in addition to their anti-inflammatory and analgesic effects, NSAIDs also have unwanted effects on the upper gastrointestinal tract (GI).

According to the FDA, the prevalence of serious events (symptomatic ulcers, bleeding, perforation) is in 1-2% of patients treated with NSAIDs for up to three months, while for those patients treated for a year, it can be seen in 2-5%.

The occurrence of GI toxicity appears to be strictly correlated to the mechanism of action of these drugs, namely the inhibition of the enzyme cyclooxygenase. In fact, inhibition of platelet cyclooxygenase, which causes prolonged bleeding time, and inhibition of cyclooxygenase in gastrointestinal mucosa, which results in a decreased synthesis of cytoprotective gastric prostaglandins, represent the major cause of serious GI toxicity (upper GI bleeding events), leading to emergency hospitalization of a number of patients taking NSAIDs.

Over the last 10 years, discovery of the second isoform of cyclooxygenase (COX-2) has led to the development of specific COX-2 inhibitors and resulted in potent anti-inflammatory compounds with significantly reduced GI toxicity. Additionally, novel compounds have been developed in the search for better-tolerated nonsteroidal anti-inflammatory drugs, by adding a nitric oxide (NO)-releasing group conventional NSAIDs.

The highlights of this symposium will focus on these new families of NSAIDs and provide important insights into the mechanisms of action as well as potential therapeutical uses of these novel anti-inflammatory agents.
Immunomodulatory effects of NO-NSAIDs and COX-2 selective inhibitors. Relevance for their anti-inflammatory activity

Classical NSAIDs are known to exert their anti-inflammatory effect mainly through the decreased production of prostaglandins resulting from inhibition of cyclooxygenase enzymes. Nevertheless, evidence has appeared in the literature suggesting that NSAIDs may exert additional activities independent from inhibition of the synthesis of prostaglandins. It has been shown that aspirin and other NSAIDs inhibit nuclear factor-kappa B (NF-kB) mobilization as well as the expression of adhesion molecules in endothelial cells and monocytes. On the other hand, little is known about the effect of currently available NSAIDs on T-lymphocytes and therefore the production of cytokines that play a central role in the evolution of the inflammatory response.

CD4 positive T-lymphocytes are known to differentiate into Th1 and Th2 upon activation with IL-2. Th1 cells mainly release proinflammatory cytokines such as interferon gamma (IFNγ), while Th2 response is characterized by the release of IL-4, IL-5 and IL-10, this later cytokine possess anti-inflammatory activities. The activation of the Th1-dependent cytokine pathway is known to be involved in the development of inflammatory pathologies such as arthritis, inflammatory bowel disease, and neurodegenerative diseases. The recent development of NSAIDs carrying a NO-donor group has opened a new field of research in anti-inflammatory therapy. In fact, nitric oxide, formerly known as endothelial derived relaxing factor (EDRF), possesses several different immunoregulatory activities:

- suppression of IL-2 and IFNγ gene expression and enhancement of IL-4 production
- increase in the production of mediators that induce Th2 differentiation in antigen-presenting cells
- induction of T-cell apoptosis
- interference with selectin-dependent cell adhesion
- inhibition of Th1-like and enhancement of Th2-like response at low, micromolar concentrations.

Furthermore it has been shown that iNOS knockout mice develop Th1-mediated autoimmune disease.

Recently it has been shown that endogenous and exogenous NO inhibits IL-1β and IL-18 release from macrophages, an effect mediated by the nitrosylation of ICE (IL-1β converting enzyme). This enzyme plays an important role in the inflammatory response because it is responsible for the processing of procytokines into the active form of IL-1β and of IFNγ-inducing factor (IGIF or IL-18). IL-1β and IL-18, synthesized by macrophages and T-lymphocytes, are potent inducers of the synthesis of additional inflammatory cytokines such as tumor necrosis factor alpha (TNFα), IFNγ, and IL-8 (Figure 1).

![ICE has a key role in inflammation](image)

ICE belongs to the family of caspases, enzymes possessing cysteine aspartate protease activity, and is active as an eterotetrameric form of two different subunits: p10 and p20. As expected from the potential role of ICE in inflammatory responses, ICE knockout mice showed decreased inflammatory responses to endotoxic shock without significant changes in thymocyte and macrophages apoptosis. Recently, it has been shown that inactive caspases exist as a nitrosylated zymogen in several unstimulated human cell lines, with nitrosylation occurring at the catalytic-site cysteine. Activation of cells causes not only cleavage of zymogen to its active subunits, but also denitrosylation, thereby
freeing the active site thiol.
Chemically reactive NO may therefore represent an effective tool for the control of ICE activity, thus modulating the associated cascade of inflammatory cytokines.
NCX-4016, a nitro derivative of aspirin, but not aspirin itself, is very effective in dose-dependently inhibiting IL1β, IL-18 and IFNγ production by LPS-activated human macrophages. Similarly, a modest but significant inhibition of TNFα and IL-8 production by NCX-4016, was also observed.
Unlike NCX-4016, specific inhibition of COX-2 is completely unable to modify LPS-induced cytokine production by monocyte-derived human macrophages, in spite of the very efficient inhibition of PGs production achieved in the same cell model of inflammatory response.
According to the potential activation of ICE during inflammatory responses, ICE activity, evaluated by the cleavage of a specific substrate peptide (YVAD), was increased upon LPS activation; pretreatment with NCX-4016, but not with the parent compound aspirin, dose-dependently decreased the LPS-induced ICE activity, suggesting a potential inhibitory effect of NCX-4016 on ICE activity.
In addition to its effect on the activity of ICE, NCX-4016 was also able to inhibit the expression of ICE, as determined by immunoblotting of the p20 subunit.
The potential nitrosylation of ICE by NCX-4016 was investigated using DTT, which is able to reduce nitrosylated proteins; co-administration of NCX-4016 with DTT significantly decreased the inhibition of ICE observed using NCX-4016 alone, suggesting that the NO-donor ability of NCX-4016 may indeed be involved in such an effect.
Additional in vivo effects of NCX-4016 were studied using an animal model of acute, T-lymphocytes-dependent hepatitis, obtained by treating mice with concanavalin A (Con-A). Pretreatment with NCX-4016 completely abolished the increase in plasma aminotransferase levels, and protected against liver necrosis induced by the treatment of mice with Con-A, as assessed by liver histology.
In accordance with these observations, NCX-4016 significantly inhibited the production of IL1β and IFNγ observed in vivo following treatment with Con-A, as well as the LPS-dependent ICE activity observed in mouse spleen in vitro.
Furthermore, liver immunohistochemistry showed that pretreatment with NCX-4016 significantly decreased Con-A-dependent expression of Fas-ligand, a member of the tumor necrosis factor (TNF) family of death-inducing ligands, involved in apoptotic processes by interaction with the death receptor Fas.
Taken together, the results presented support the hypothesis that NCX-4016, and possibly other members of the novel family of NO-NSAIDs, not only exert COX-dependent and COX-independent anti-inflammatory activities, but as a consequence of the NO-donor ability of these compounds, they can exert additional anti-inflammatory activities related to ability to interfere with the caspase 1-dependent pathway of inflammation.

♦ Caspase 1 (ICE), an enzyme which possesses cysteine aspartate protease activity, has a key role in inflammatory processes involving the production of IL-1β and IFNγ-inducing factor (IGIF or IL-18) being responsible of the conversion of procytokines into their active forms.
♦ NCX-4016, a nitro-derivative of aspirin, but not aspirin or selective COX-2 inhibitors, inhibits the LPS-induced production of different cytokines.
♦ NCX-4016 inhibits the activity of ICE both “in vitro” and “in vivo”, possibly through the nitrosylation of ICE protein.
♦ NCX-4016, but not aspirin, protects experimental animals from T-cell-dependent acute liver injury.
♦ NCX-4016 inhibits prostaglandin (PG) production in experimental animals “in vivo”, without causing gastric lesions associated with the treatment with aspirin.
Development and marketing of selective COX-2 inhibitors have provided a new family of GI safer NSAIDs; nonetheless it must be pointed out that some issues concerning the mechanism of GI toxicity induced by NSAIDs need further clarification in order to achieve a correct picture of the role of COX-1 and COX-2 in the GI system.

The first issue to be addressed is the hypothesis that inhibition of gastric COX-1 is responsible for the GI toxicity of NSAIDs. The results obtained with COX-1 knockout mice showed that, in spite of negligible gastric PG synthesis, mice did not show spontaneous gastric damage, although still sensitive to indomethacin-induced hemorrhagic erosions. Furthermore the highly specific COX-1 inhibitor developed at Searle (SC-560), did not cause gastric damage in rat when administered at a dose able to completely suppress TXB$_2$ formation in whole blood ex vivo, as well as to significantly inhibit gastric PGE$_2$ synthesis. Similarly, a COX-2 selective inhibitor (celecoxib) at a dose capable of completely suppressing PGE$_2$ production in inflammatory exudates, did not cause either inhibition of gastric PG production or gastric damage. Interestingly, co-administration of the two drugs, resulting in suppression of both COX-1 and COX-2 activity, caused gastric damage comparable to that obtained with the conventional NSAID indomethacin (Figure 2).

Evaluation of ketorolac for COX-1 and COX-2 selectivity in vivo showed that at 1-3 mg/kg the drug almost completely suppressed COX-1 activity without affecting COX-2 activity; interestingly, gastric damage became evident only at higher doses (10-30 mg/kg) in parallel with increasing inhibition of COX-2 activity. As previously observed for SC-560, co-administration of ketorolac and a specific COX-2 inhibitor at doses which did not cause GI damage, resulted in a striking increase in the number of gastric lesions.

To determine the mechanism underlying mucosal injury that requires both COX-1 and COX-2 inhibition, reduction of gastric blood flow and increased adherence of leukocyte within the gastric microcirculation were evaluated in vivo by laser-Doppler flowmetry and intravital microscopy. The results obtained showed that treatment with either indomethacin or a COX-1 inhibitor, but not with the COX-2 specific inhibitor, significantly decreased gastric blood flow, suggesting that this is a COX-1-mediated effect. On the other hand, leukocyte adhesion was significantly increased by indomethacin and the COX-2 inhibitor, but not by the COX-1 specific inhibitor, highlighting the involvement of COX-2 in the mechanism which leads to enhanced leukocyte adhesion (Figure 3).
These results are consistent with the information obtained from COX-1 knockout mice, and therefore support the notion that gastric damage induced by NSAIDs requires inhibition of both COX-1 and COX-2. In light of the observed effect of NSAIDs on leukocyte adhesion and vascular blood flow, it is of interest to evaluate the effect of these compounds in pathological conditions characterized by stimulated vascular adhesion and decreased blood flow such as ischemia-reperfusion injury and hemorrhagic shock. Recent results showed that COX-2 inhibitors are able to aggravate ischemia-reperfusion injury in the rat stomach, in spite of the fact that COX-2-dependent production of PGs in the gastric mucosa could not be detected. Several groups have also reported that COX-2 is expressed at the margins of stomach ulcers, where the healing process is taking place, and that treatment with COX-2 inhibitors induces a significant retardation of ulcer healing, suggesting involvement of COX-2 in the healing process. Several NO-NSAIDs have been tested for the potential of delaying ulcer healing, but it has been shown that, unlike the parent compounds, they accelerate rather than delay the healing process (Figure 4).

In a model of experimental colitis, PG synthesis was increased more than 25-fold, and repeated administration of a COX-2 selective inhibitor exacerbated the colitis, leading to perforation and death of the animals, similar to the results obtained in COX-1 and COX-2 knockout mice, where colitis was worsened when compared to wild-type animals. Using the same experimental colitis model, diclofenac showed similar results while NO-diclofenac did not cause an increase in the mortality, suggesting that the NO-donor ability of NO-NSAID might protect against this important side effect of NSAIDs.

Among the important clinical side effects associated with the treatment with NSAIDs it has been shown that NSAIDs exacerbate hypertension and reduce efficacy of antihypertensive therapy especially in the elderly. Recently COX-2 inhibitors have been shown to significantly affect prostacyclin production in humans in vivo, therefore potentially affecting the antiaggregating and antihypertensive effect of this potent arachidonic acid metabolite.

In agreement with this hypothesis, the COX-2 selective inhibitor rofecoxib has been shown to cause a significant increase in the incidence of hypertension; nevertheless the results of a large study on the incidence of adverse affects associated with the assuption of celecoxib vs NSAIDs did not show evidence of an increased risk of hypertension or peripheral edema in subjects taking this specific COX-2 selective inhibitor.

A significant increase in blood pressure was observed in rats treated for up to three weeks with an anti-inflammatory dose of celecoxib, either in normal rats or in rats in which hypertension had first been induced by addition to the drinking water of an inhibitor of NO synthase. Very different from this was the behaviour of NCX-4016, the nitro-derivative of aspirin, that not only did not cause an increase in blood pressure, but completely reversed the hypertensive effect observed in rats treated with the NO-synthase inhibitor. It is also important to note that blood pressure evaluation was performed 20 hours after the last treatment with NCX-4016, and cannot be referred to the acute effect of the NO-donor moiety present within the structure of NCX-4016.

Although an effect of NO-donor compounds might have been anticipated in a model of NO-dependent hypertension, NO-NSAIDs proved to be different from conventional NSAIDs also in a model of hypertension not related to a deficit of NO synthesis; in fact, treatment with naproxen resulted in a significant increase of the hypertensive response.
Gastrointestinal and cardiovascular safety of COX-2 inhibitors and NO-NSAIDs

Evaluation of GI toxicity in experimental animals shows that gastric damage induced by NSAIDs requires inhibition of both COX-1 and COX-2. Selective COX-2 inhibition aggravates gastric ischemia-reperfusion injury, delays ulcer healing, and aggravates experimental colitis in the rat. Selective COX-2 inhibition increases the severity of GI damage induced by aspirin. NO-NSAIDs compared to their parent compounds neither delay ulcer healing nor aggravate experimental colitis. NO-aspirin is more potent than the parent compound aspirin in inhibiting thrombin-induced platelet aggregation "in vitro", but does not cause the significant gastric damage observed with increasing doses of aspirin. NO-donors reduce the risk of upper GI bleeding associated with the use of NSAIDs.

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Effect of partial renal stenosis in rats, while NO-naproxen not only did not worsen the hypertension caused by this procedure, but indeed significantly decreased, the blood pressure measured after one and two weeks of treatment, when compared to untreated. No direct hypotensive effect of was observed upon i.v. infusion of high doses of NO-NSAIDs in normotensive animals. Concerning the use of aspirin for the reduction of the incidence of myocardial ischemia and stroke, a therapeutic effect that is not shared by COX-2 inhibitors. NO-aspirin was shown to be approximately 7-fold more potent that the parent compound in the inhibition of thrombin-induced human platelet aggregation in vitro. Interestingly this effect was not associated to an increase in bleeding time.

GI safety was assessed in experimental animals, showing that unlike the parent compound, NO-aspirin did not cause GI damage up to doses of 300 mg/kg. More interestingly it has recently been reported that the risk of upper GI bleeding in subjects taking aspirin together with NO-donor compounds did not differ from control subjects, and was significantly lower than the risk observed in both NSAIDs and prophylactic aspirin users (Figure 5).

In conclusion, focusing on pre-existing GI ulceration and ischemia-reperfusion injury, it has been possible to collect evidence that COX-2 inhibitors may not be as safe as in most situations, and that NO-NSAIDs may differentiate from COX-2 inhibitors. Combination therapy of prophylactic aspirin and anti-inflammatory COX-2 inhibitors has recently been shown to result in significant GI toxicity both in animals and in man. Additional clinical trials are needed to clearly establish the safety of these compounds.

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Colorectal cancer causes over 500,000 deaths per year worldwide and represent one of the main cause of death by cancer in the U.S., as well as in Italy. Although the absolute figures are actually decreasing, population aging may cause a rebound in the number of deaths, in light of the fact that over 93% of the cases of colorectal cancer occur after the age of 50. Much data has been collected and population-based studies of different types conducted that indicate that use of aspirin or other NSAIDs leads to a 50% reduction in mortality, and, in some studies, risk, of colorectal cancer. Over 35 studies have indicated the occurrence of such an effect, specifically in the reduction of adenoma and carcinoma incidence as well as cancer-associated mortality. These activities have been observed across different cohorts of the general population, as well as across different groups of at-risk subjects, and decreased the risk in both men and women irrespective of age, left- or right-sided lesions of the colon, with extraordinarily consistent results observed in the U.S., Europe, and Australia. Results from a clinical trial showed that 150 mg twice/day Sulindac, a traditional NSAID, regresses size and number of colorectal adenomas in patients with familial adenomatous polyposis (FAP), an inherited form of colorectal cancer. Suspension of treatment after nine months resulted in adenomas growing back to their original size and number, (Figure 6) suggesting that NSAID may indeed affect the biology of these premalignant lesions.

In light of these epidemiological and clinical research data it was of interest to assess the expression of the two different isoforms of COX, namely COX-1 and COX-2, in these tumors. Previous data on relative COX-1 and COX-2 distribution obtained in normal gastrointestinal tract showed COX-1 is expressed throughout the entire GI tract, while COX-2 is almost undetectable in most GI tissues. Characterization of over 150 individual colorectal adenocarcinomas demonstrated a significant elevation of COX-2 expression at the mRNA in 85% of the cases, while the same was true in approximately 50% of over 40 individual adenomas. Additional studies looking at the protein expression by immunohistochemistry and immunoblotting showed that in the adenocarcinoma there is always a 2- to 50-fold increase in COX-2 expression, while normal mucosa do not show COX-2 expression. Expression of COX-2 mainly takes place in epithelial cells, but significant expression may also take place in the stroma, depending on the age and differentiation of the tumor.

An important question needing to be addressed is what leads to the increased expression of COX-2 in these tumors and, knowing that RAS mutation takes place in 60-70% of these tumors, studies were carried out to determine if transfection of mutated RAS would induce COX-2 expression. The results showed that transfection of mutated RAS gene into nontransformed rat intestinal epithelial cell was able to induce significant expression of COX-2 both at the mRNA and protein level, possibly through the induction of a protein that stabilizes COX-2 mRNA by binding to its 3' untranslated region. Furthermore, other genes involved in the progression of normal colonic epithelial cell in colorectal cancer, such as SRC, can also induce COX-2 expression.

It has been shown that human colonic adenocarcinoma cell lines such as HCA-7 cells, obtained from a rectal carcinoma, constitutively express Cyclooxygenase-2: a target for cancer treatment and prevention.
COX-2 and synthesize large amounts of prostaglandins. When these cells were grown as xenografts in nude mice, selective COX-2 inhibition dramatically reduced by 80-90% the volume of the tumor (Figure 7).

COX-2 gene knockout or selective COX-2 inhibition showed a significant reduction in the number and size of the intestinal polyps in Apc-Δ716 knockout mice, a model of human familial adenomatous polyposis, providing direct genetic evidence that COX-2 plays a key role in tumorigenesis. Selective expression of COX-2 has also been observed in azoxymethane-induced colonic tumors in rats, and inhibition of COX-2 resulted in a very significant reduction in both invasive and noninvasive adenocarcinomas. Significant inhibition of tumor volume deriving from Lewis lung carcinoma (LLC) cells implanted as isograft into B6 mice, occurred using a selective COX-2 inhibitor, showing that COX-2 inhibition could result effective in tumor models different from colorectal cancer. Furthermore, deletion of the COX-2 gene in the host animals affected tumor growth more significantly than use of the COX-2 inhibitor, while deletion of COX-1 did not cause any effect, underscoring the key role played by COX-2 in the development of these very aggressive tumors. Evaluation of vascular density of tumors observed in COX-2 wild-type and null mice showed that wild type had a more significantly increased vascularization than the COX-2 null mice, in agreement with the literature suggesting an involvement of COX-2 in angiogenesis. A key to the interpretation of the effects on tumor vascular density observed in COX-2 null mice, was provided by evaluation of the production of vascular endothelial growth factor (VEGF) by stromal fibroblasts obtained from wild type, COX-1, and COX-2 knockout mice: while COX-1 knockouts have a production of VEGF that is comparable to controls (WT), COX-2 null mice show very little production of this important angiogenic factor (Figure 8).

Furthermore, a selective COX-2 inhibitor (SC-58125) was able to inhibit the production observed in wild type animals in a concentration-dependent fashion. Several reports have shown that expression of COX-2 in epithelial cells makes them resistant to apoptosis and enhance their metastatic potential. Together with the observed effect on angiogenesis, these results suggest that COX-2 may be a relevant target for the prevention and/or the treatment of cancer, and that appropriate clinical trials to test this hypothesis should be undertaken.

Recently, together with enhanced COX-2 expression, it has been reported that adenocarcinomas present enhanced expression of peroxisome proliferator activating receptor delta (PPARδ). Using an artificial model where the ligand binding domain of PPARδ was cloned into a reporter system in the presence of transfected COX-2 and prostaglandin I synthase (the enzyme converting the endoperoxide PGH₂ into prostacyclin), a very significant transactivation of PPARδ was observed; conversely, the activation was completely prevented by treatment with a specific COX-2 inhibitor.
It must be noted that the transactivation observed under these condition was significantly higher than that observed with specific PPARδ ligands, and may represent evidence that a prostanoid is the relevant endogenous ligand for this nuclear hormone receptor.

The use of aspirin and other NSAIDs leads to a 50% reduction in mortality from colorectal cancer without significant differences with respect to sex, age, or geography.

Expression of COX-2 is significantly elevated in colorectal adenocarcinomas.


COX-2 gene knockout or selective COX-2 inhibition, both showed a significant reduction in the number and size of the intestinal polyps in Apc-Δ716 knockout mice, a model of human familial adenomatous polyposis.

COX-2 gene knockout, but not COX-1 gene knockout, caused significant reduction in tumor volume in Lewis lung carcinoma isografts.

COX-2 may play a role in tumor vascularization, as shown by reduced vascular density in tumors and reduced synthesis of VEGF in COX-2 knockout mice.

COX-2 may be a relevant target for prevention and/or treatment of colorectal cancer.
Bone resorption represents an important feature in the pathogenesis of bone disease. In osteoporosis, a pathological condition affecting 30% of women and 12% of men, enhanced bone resorption leads to loss of bone mass and fractures. Enhanced bone resorption causing bone deformity, bone pain, and ultimately bone fractures, is also observed in Paget's disease, a different pathology that affects 3% of the elderly population. The costs associated to these pathologies are over 600 million dollars per year in the U.K., as well as in most western countries. Prostaglandins are likely to play an important role in the physiologic and pathologic responses of skeletal tissue. They are potent agonists that can stimulate bone. In vivo, the major effect of exogenous prostaglandins, particularly prostaglandin E₂, is to stimulate resorption and formation. These effects appear to be mediated at least in part by cyclic 3', 5'-adenosine monophosphate. Prostaglandins can inhibit the activity of isolated osteoclasts, probably also by a cyclic 3', 5'-adenosine monophosphate-mediated mechanism. Prostaglandin production by bone cells is regulated by mechanical forces, cytokines, growth factors, and systemic hormones. Regulation is associated with marked changes in the "inducible" prostaglandin G/H synthase COX-2.

Based upon the evidence of the involvement of prostaglandins (PG) in bone resorption process, the prophylactic use of NSAIDs may prove to be beneficial in preventing bone pathologies. Unfortunately the use of NSAIDs often causes severe GI side effects, as a result of reduced blood flow in the stomach subsequent to inhibition of PG synthesis. Evidence showing that NO-NSAIDs do not cause GI side effects, possibly because of the vasodilatory effects of NO, suggests that they may represent a valid alternative to traditional NSAIDs for the prevention of bone resorption.

Nitric oxide possesses additional activities on bone metabolism; at low concentrations it may induce bone resorption, but at higher concentrations (>30 nM nitrate over 24 hours, in vitro) it actually causes effective inhibition of bone resorption, as indicated by the release of radiolabeled Ca⁺⁺ from mouse calvarial organ cultures. A nitro derivative of flurbiprofen (compound HCT-1026) has been evaluated in an in vitro model of osteoblast-osteoclast co-culture assay. In this model osteoblasts, bone forming cells, are cultured with a source of osteoclasts, such as spleen cells, on a dentin slice, and either the number of osteoclasts or directly bone resorption pit formation under reflected light microscopy is evaluated. Both basal and IL-1β induced osteoclast formation and resorption area were significantly inhibited by HCT-1026, while flurbiprofen had a minor effect only on IL-1β stimulated bone resorption (Figure 9).

Interestingly, this in vitro effect of HCT-1026 was not mimicked by co-administration of flurbiprofen and SNAP, a specific NO-donor compound, raising the question if the observed effect on bone resorption was indeed resulting from NO released from the nitro group present in the structure of HCT-1026. The activity of HCT-1026 in vivo was evaluated in a model of postmenopausal bone loss induced by ovariectomy in the mouse, where bone density of tibia was quantitated by peripheral computer tomography. Prostaglandins are believed to play a role in postmenopausal bone loss because estrogen deficiency, which increases bone turnover, can also increase prostaglandin production in bone.

In trabecular and cortical bone of sham operated animals HCT-1026 and flurbiprofen both caused a minor loss of bone, possibly related to the effects of prostaglandins on osteoblasts. On the contrary, in ovariectomized animals HCT-1026,
but not the parent compound flurbiprofen, caused a significant reduction of both trabecula and cortical bone loss (Figure 10). HCT-1026 may be of therapeutic value in the clinical practice, perhaps in cytokine-induced bone loss such as that observed in rheumatoid arthritis, or in postmenopausal bone loss. HCT-1026 has proved to be a very potent inhibitor of osteoclastic activity in vitro, being in molar terms as potent as bisphosphonates. Furthermore it protects against ovariectomy-induced bone loss in rodents and most of its effects cannot be mimicked by co-administration of the parent NSAID flurbiprofen and a NO-donor compound, raising the hypothesis that this molecule represents a novel pharmacophore with very important effects on osteoclastic activity.

- Bone disease such as osteoporosis has important financial and clinical implications in western countries.
- The effect of NO on bone resorption depends on concentration and on the presence of other local factors.
- HCT-1026, a nitro derivative of the NSAID flurbiprofen, is more potent than the parent compound as inhibitor of osteoclast formation induced by IL1β as well as inhibitor of IL1β-induced bone resorption “in vitro”.
- HCT-1026, but not flurbiprofen causes apoptosis in mature rabbit osteoclasts “in vitro” and protects against ovariectomy-induced bone loss in mice “in vivo”.
- HCT-1026 may be of therapeutic value in the prevention of cytokine-induced and postmenopausal bone loss.
Conclusions

COX-2 inhibitors have proved to exert anti-inflammatory effects with reduced GI toxicity, while NO-NSAIDs, originally described as GI-sparing anti-inflammatory drugs, may represent a new class of therapeutic agents with additional mechanisms of action and significantly enhanced efficacy. Evidence that COX-2 inhibitors, under specific conditions may not have the same efficacy as traditional NSAIDs suggests that either COX-1 in some cases contributes to the production of hyperalgesic/inflammatory prostaglandins or that there are COX-independent effects. Increasing evidence is available that COX-2 inhibitors may be beneficial in the prevention of colorectal cancer. Similarly, the NO-NSAIDs seem very promising in this regard. The fact that a large dose of a COX-2 inhibitor was necessary to produce a significant reduction of polyps in Familial Adenomatous Polyposis (FAP) suggests effects on COX-1 or COX-independent activities may account for the observed effect. Addition of the NO-donor group to the aminosalicylic acid resulted in a tremendously increased anti-inflammatory efficacy in inflammatory bowel disease; similarly NO-acetaminophen proved to have enhanced analgesic activity, reduction of hepatic toxicity, and additional anti-inflammatory activity not present in the parent compound. All the contributions of the symposium have raised important questions that need to be addressed by appropriate clinical trials, in order to clearly assess the safety and the efficacy of cyclooxygenase-2 inhibitors and NO-NSAIDs for their use in inflammation, cancer, as well as additional potential therapeutic uses.


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