Development of NOSH-NSAIDs: a new class of anti-inflammatory pharmaceuticals for the treatment of cancer

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Non-steroidal anti-inflammatory drugs (NSAIDs) have anti-cancer (chemopreventive) properties; however, side effects preclude their long-term use. NOSH-NSAIDs, designed as safer alternatives, are novel hybrid chimaeras that release nitric oxide (NO) and hydrogen sulfide (H2S). NOSH-NSAIDs are gastrointestinally safe yet retain all the pharmacological properties of their native NSAID. NOSH-NSAIDs are orders of magnitude more potent than their conventional counterparts in inhibiting the growth of various human cancer cell lines of different tissue origins, adenomatous, epithelial and lymphocytic. This growth inhibition is a result of a reduction in cell proliferation and cell cycle arrest, leading to increased apoptosis. In xenograft mouse models of cancer, NOSH-aspirin was better than normal aspirin as a chemopreventive agent; it dose-dependently inhibited tumour growth and tumour mass. NOSH-naproxen was significantly more efficacious than normal naproxen in reducing the growth of established tumours.

Inflammation and cancer

Inflammation as a fundamental response to injury has been recognized for thousands of years. The Egyptians described abscesses and ulcers, and the Code of Hammurabi (2000 BC) details instructions on how to treat abscesses of the eye. The first comprehensive description of inflammatory symptoms can be found in De Medicina, written by Aulus Celsus (~25 BC–AD 38) who described the four symptoms of inflammation as rubor, tumor, color and dolor (redness, swelling, heat and pain). The fifth sign of inflammation, functio laesa (impaired function) was added by Galen of Pergamon, a physician to the Roman Emperor Marcus Aurelius, some 100 years later. However, Hippocrates may have been the first physician to regard inflammation as the beginning of a healing process, introducing words such as oedema and erysipelas to describe its symptoms.

Whereas acute inflammation is an adaptive host defence mechanism against infection or injury and is self-limiting, chronic inflammation may lead to various ailments including cancer. Noticing the presence of leukocytes in neoplastic tissues, Rudolf Virchow in 1863 proposed that there was a functional relationship between inflammation and cancer. Since then, a considerable body of evidence supports the notion that tumours can originate at the sites of infection or chronic inflammation. For example, inflammatory diseases increase the risk of developing bladder, cervical, gastric, intestinal, oesophageal, ovarian, prostate and thyroid cancer.

Evidence that NSAIDs protect against cancer

NSAIDs are primarily used: (i) as analgesics to relieve the most common forms of pain, (ii) as anti-pyretics to reduce fever, (iii) as anti-inflammatory agents and (iv) as anti-thrombolytics – the so-called four ‘As’. A key piece of evidence that links cancer and inflammation is that the use of NSAIDs such as aspirin reduces the risk and mortality from many cancers. Over 30 epidemiological studies, collectively describing results on more than 1 million individuals, have established NSAIDs as the prototypical chemopreventive agents against many forms of cancer. In particular, three well-designed randomized, double-blind trials of aspirin as a chemopreventive agent against colorectal adenomas established its chemopreventive effect. These studies have expanded to underscore the chemopreventive effects of NSAIDs in general against colon, breast, pancreas, bladder, head and neck, oesophageal, ovarian, prostate, hepatocellular and skin cancers. Of significance are two relatively recent reports indicating that daily aspirin use, whether regular strength or low dose, not only resulted in reductions in cancer incidence and mortality, but also prevented distant metastasis.

The general concept of cancer chemoprevention by NSAIDs cannot be overstated. However, all studies to date have failed to provide details of the big picture. For example, which one of the nearly 30 NSAIDs is the most effective? What is the optimal dose and what is the ideal
schedule of administration? Even if such information existed, it would probably be of limited or no practical value. The reason is that, although we have unassailable proof-of-principle for chemoprevention, current NSAIDs cannot overcome two prohibitive limitations concerning their safety and efficacy. For example, for colon cancer, the one most thoroughly studied, NSAIDs can prevent at best 50% of the cases; all NSAIDs eventually cause some degree of gastrointestinal (GI) erosion that may eventually lead to ulcers, with most having cardiovascular and renal side effects.

Side effects of NSAIDs limits their long-term use

Although the use of NSAIDs in general, and aspirin in particular, as a chemopreventive agent is highly convincing, the use of these drugs is limited by their significant toxicity, which largely fall into three areas: GI, ranging from dyspepsia to GI bleeding, obstruction and perforation, and renal and cardiovascular. It is estimated that about 16,500 NSAID-related deaths occur among patients with rheumatoid arthritis or osteoarthritis every year in the United States. This figure is greater than the number of deaths from multiple myeloma, asthma, cervical cancer or Hodgkin’s disease. If deaths from GI toxic effects from NSAIDs were tabulated separately in the National Vital Statistics reports, these effects would constitute the 15th most common cause of death in the United States. Yet these toxic effects remain largely a ‘silent epidemic’, with many physicians and most patients unaware of the magnitude of the problem.

The search for better NSAIDs

Many different approaches have been attempted in this area, the most notable being the generation of selective cyclooxygenase-2 (COX-2) inhibitors or coxibs. In patients with a low risk for GI damage, coxibs have been successful in the short term in limiting the upper GI ulceration associated with traditional NSAIDs. However, in patients with co-morbidities or other factors that increase the risk of GI ulceration, the benefits of coxibs over traditional NSAIDs is significantly reduced. Several large-scale clinical trials have shown that long-term use of coxibs and even traditional NSAIDs is associated with an increased risk of adverse myocardial events. Also, COX-2 inhibition in the kidneys could lead to increases in blood pressure and hence increases in myocardial infarctions and stroke. These major side effects have resulted in a number of coxibs being withdrawn from the marketplace and some have suggested that there might be a ‘class effect’ associated with coxibs. Currently, all coxibs and traditional NSAIDs are required by the FDA (Food and Drug Administration) to carry a black-box warning.

The concept of NOSH-NSAIDs

In our search for better and safer NSAIDs that would overcome the limitations of traditional NSAIDs, we developed NOSH-NSAIDs. These are hybrid molecules consisting of a traditional NSAID backbone to which NO and H\textsubscript{2}S-releasing moieties have been covalently attached, Figure 1 shows an example using aspirin as the backbone. We knew that by inhibiting the enzyme COX, NSAIDs reduce prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and prostacyclin (PGI\textsubscript{2}) both of which are known to be beneficial or protective to the GI mucosa. Thus, reduced levels of these prostanoids would in part be responsible for the notorious GI side effects of NSAIDs manifested as breakdown of mucosal defence mechanisms (leukocyte adherence, decreases in blood flow, bicarbonate and mucus secretions). We also knew that NO and H\textsubscript{2}S have some of the same properties as prostaglandins within the gastric mucosa. For example, NO and H\textsubscript{2}S increase blood flow, which reduce the effects of luminal irritants. Both also increase mucus and bicarbonate secretions thus modulating other components of the mucosal defence systems. Therefore, coupling NO and H\textsubscript{2}S-releasing moieties to an NSAID might deliver NO and H\textsubscript{2}S to the site of NSAID-induced damage, thereby decreasing gastric toxicity. This rationale has proved to be quite successful. Another consideration was that NOSH-NSAIDs might also prove to have enhanced...
cardiovascular and renal safety profiles because NO and H\textsubscript{2}S have protective roles in the cardiovascular and renal systems. We also considered the possibility that there could be synergy between NO and H\textsubscript{2}S thus increasing potency and perhaps efficacy.

**NOSH-NSAIDs have enhanced GI safety profiles**

We used an acute model of ulcerogenesis to compare the effects of three traditional NSAIDs, aspirin, naproxen and sulindac, to their NOSH counterparts. We chose these three NSAIDs because aspirin is used extensively worldwide not just as a ‘painkiller’ but for its fever and blood-clot-reducing properties and is used in patients who have had a heart attack, it has also been shown to have chemopreventive properties. Naproxen is mainly used as a potent anti-inflammatory and is employed in the management of osteoarthritis, but is known to have significant GI toxicity. Sulindac is used in the management of patients with familial adenomatous polyposis (FAP), a disease which is characterized by hundreds of colorectal adenomatous polyps that eventually progress to colorectal cancer; sulindac use is limited by its toxicity, which can affect up to 20% of patients.

Rats treated with just the vehicle had a normal glandular region on the surface of their stomach, i.e. no ulcerative damage. However, administration of aspirin, naproxen or sulindac resulted in extensive mucosal injury, depicted as bleeding ulcers. Unlike these NSAIDs, treatment with NOSH-aspirin, NOSH-naproxen or NOSH-sulindac at equimolar doses did not produce any significant ulcerative damage, representing a remarkable enhancement in GI safety (Figure 2). This observation is in line with the protective roles of NO and H\textsubscript{2}S within the gastric mucosa.

**NOSH-NSAIDs – basic pharmacological properties**

In the United States, over 30 million people use NSAIDs on a daily basis, therefore a large number of people can potentially be subjected to untoward effects. Having established that NOSH-NSAIDs were GI safe/friendly we then evaluated these novel chimaeras against their respective native NSAIDs in various \textit{in vivo} settings addressing the four ‘As’.

We compared the anti-inflammatory properties of aspirin, naproxen and sulindac, to that of NOSH-aspirin, NOSH-naproxen and NOSH-sulindac in rats. In general, all three NSAIDs and their respective NOSH counterparts caused a significant reduction in inflammation. Results for aspirin versus NOSH-aspirin are shown in Figure 3, we have reported similar results for NOSH-naproxen and NOSH-sulindac.
It is well known that NSAIDs exert a moderate anti-pyretic effect when administered orally. We therefore wanted to determine the decrease in body temperature induced by NOSH-NSAIDs compared with that obtained with NSAIDs (also in rats). Qualitatively similar results were obtained for all NSAIDs/NOSH-NSAIDs, with the results for aspirin and NOSH-aspirin shown in Figure 4A.

NSAIDs in general are the mainstay in the treatment of pain. In that respect, we compared the analgesic effects of NOSH-NSAIDs with that of their respective NSAIDs in an in vivo model of hyperalgesia. Both were equally effective in increasing the threshold response to painful stimuli, the results for aspirin and NOSH-aspirin are shown in Figure 4B. NOSH-aspirin was recently shown to have greater potency than aspirin in reducing inflammatory pain in several clinically relevant models. The enhanced pain blocking effect of NOSH-aspirin appeared to be due to its ability to reduce the production of pain-inducing cytokines such as IL-1β. NOSH-aspirin was also shown to reduce hyperalgesia (increased sensitivity to pain), caused by direct-acting hyperalgesic mediator in a mechanism dependent on modulation of potassium channels.

Aspirin is the only NSAID that is commonly used to prevent a secondary heart attack. We therefore compared the anti-thrombotic effects of aspirin and NOSH-aspirin using collagen-induced platelet aggregation of human platelet-rich plasma (PRP). The results, expressed as IC50, are shown in Figure 4C. The data does not show any statistical differences between aspirin and NOSH-aspirin. Although naproxen and sulindac are seldom if ever used for this purpose, we have shown that they were qualitatively equivalent to their NOSH-NSAID counterpart as an anti-thrombotic agent.

Using NOSH–NSAIDs to target cancer

Studies evaluating the effect of NOSH-NSAIDs on the growth of a variety of cancer cells showed that they are much more potent than the corresponding traditional NSAIDs at inhibiting the growth of these cultured cancer cells. Chemopreventive properties of the two types of NSAIDs were evaluated using colon, breast, pancreas, prostate and lung cell lines, as well as those from T-cell leukaemia. For NOSH-sulindac the potency enhancement over sulindac ranged from 1,000–10,000-fold; for NOSH-naproxen the increase over naproxen was 16,000–34,000-fold; and for NOSH-aspirin the potency enhancement over aspirin was approximately 16,000–100,000-fold. After 72 hours in colon cancer cells, this enhanced potency increased to about 250,000-fold. Although these NOSH-NSAIDs exhibited potent anti-cancer activity profiles, they minimally affected normal cells. It is important to note that these studies strongly suggest that the actions of NOSH-NSAIDs appear to be a generalized property (i.e. their effect is tissue-type independent) and that NOSH-aspirin is the most potent hybrid.
Mechanistically, using HT-29 colon cancer cells as a model, the cell growth inhibition exhibited by NOSH-NSAIDs was a result of a reduction in cell proliferation and cell cycle arrest (G$_0$/G$_1$ or G$_2$/M), leading to increased apoptosis.

However, the underlying mechanism(s) for the enhanced potency of NOSH-NSAIDs is not apparent and there is no information available regarding the kinetics of NO and H$_2$S release and their potential interactions. What is certain is that both NO and H$_2$S contribute towards the potency of the intact molecules. This is based on our earlier observations where we showed that the biological activity of aspirin plus SNAP (S-Nitroso-N-acetyl-penicillamine, which releases NO) plus ADT-OH (5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione, which releases H$_2$S) was not the same as the biological activity of the intact NOSH-aspirin molecule. Thus, the sum of parts did not equal the whole. These molecular aspects need further investigation as it has recently been reported that NO can react with H$_2$S to produce HSNO, which is a highly reactive intermediate.

In a mouse model of colon cancer, NOSH-aspirin was shown to be significantly more effective (around 5-times more potent) at reducing tumour growth and mass than aspirin alone, without the adverse GI effects of aspirin. Clearly, as a chemopreventive agent, NOSH-aspirin is superior to aspirin both in terms of efficacy and safety. These results further strengthen our original hypothesis that incorporating NO and H$_2$S-releasing moieties within the aspirin molecule would enhance both its activity/potency and safety profiles.

Similarly, studies with NOSH-naproxen versus naproxen using an in vivo xenograft mouse model of colon cancer showed that NOSH-naproxen treatment resulted in large reductions in tumour volume and mass. The decrease in tumour size and mass was associated with inhibition of cell proliferation and induction of apoptosis. The dose of NOSH-naproxen used in this mouse model was well tolerated with no apparent harmful side effects or overall gross toxicity; but all the
mice given naproxen alone died by the end of the second week of treatment from what appeared to be GI bleeding and damage to other organs such as the spleen and liver.

**Future directions**

Our studies have established three key features of NOSH-NSAIDs: (i) NO and H$_2$S are both required for the enhanced potency of NSAIDs for inhibiting cancer cell growth, (ii) this enhanced potency is manifested in cancers of varied tissue origin and (iii) NOSH-aspirin is consistently the most potent NOSH-NSAID regarding these properties. In our proof-of-concept animal studies, we have demonstrated that NOSH-NSAIDs are essentially devoid of any GI side effects even though they reduce gastric tissue prostaglandin E2 levels. The hybrid molecules retain all the positive pharmacological attributes of their respective parent NSAID. That is, they have potent anti-inflammatory, analgesic, anti-platelet and anti-platelet activities. In addition to their GI safety, NOSH-NSAIDs might also prove to have enhanced cardiovascular and renal safety profiles due to the released NO and H$_2$S. NOSH-NSAIDs are potentially useful as chemopreventive and/or chemotherapeutic agents against many types of cancer. A cartoon summarizing the classic pharmacological effects of NOSH-aspirin is depicted in Figure 5.

Current work in my laboratory is directed towards understanding the mechanisms of action of these novel compounds, focusing on molecular targets that are relevant to inflammation and cancer, and to possible interactions between NO and H$_2$S in producing a new signalling entity. These unique agents are a new class of anti-inflammatory pharmaceuticals and we are focusing on developing these for various patient applications. For example, our lead compound, NOSH-aspirin (NBS-1120) is being developed for use against a number of human malignancies. Other agents, such as NOSH-naproxen (AVT-219), are under active investigation for the management of pain and as an anti-inflammatory in the treatment of osteoarthritis.

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**Further reading**