

Innovations

Send in the gas NicOx S.A.

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Nitric oxide (NO) is a molecule of surprises: a gas that is a biological signaling molecule; a radical that is an essential regulator of numerous cell types; and a pollutant that has been the subject of over 30,000 papers in biology and medicine. How can a start-up company possibly tackle such an unwieldy subject area?

The contradictions of NicOx S.A. (Sophia-Antipolis, France) match those of its subject matter. A French company, NicOx is headed by two Italians and an American. With just 20 employees it is already overseeing 10 clinical trials (ongoing or imminent) and coordinating over 50 research collaborations. And its primary promise is that it will beat out a class of drugs — the cyclooxygenase 2 (Cox2)-specific non-steroidal anti-inflammatory drugs (NSAIDs) — that were greeted with the biggest fanfare that the pharmaceutical industry has seen for the last 20 years. The history of NO is not exactly lacking in dramatics, but if NicOx has their way the climax will be the triumph of NO as a therapeutic for everything from pain and inflammation to cardiovascular disease, Alzheimer's disease and osteoporosis.

From Alfred to That Prize

The bizarre story of NO research starts in the 1890s with Alfred Nobel. Nobel made a large part of his fortune by realizing that he could pack the highly unstable explosive nitroglycerin into a silica base to produce dynamite. But on his death bed he was confronted with nitroglycerin in another form. As he

wrote to a friend: "Isn't it the irony of fate that I have been prescribed nitroglycerin, to be taken internally! They call it Trinitrin, so as not to scare the chemist and the public." Nobel was suffering from angina pectoris — chest pain caused by insufficient blood supply to the heart — and nitroglycerin was then, and remains today, an effective treatment. It works by dilating the heart's vessels to allow greater blood flow. Nobel was not convinced and declined his doctor's offer.

Angina was far from the mind of Ferid Murad (University of Virginia, Charlottesville) in 1977. He was simply looking for chemicals that would shut off various interfering enzymes so that he could study his favorite — guanylyl cyclase (GC). But by chance one of the chemicals turned on GC, and suddenly Murad had the first known activator of this mysterious enzyme. Other activators followed, including nitroglycerin.

A toxic gas may improve the most widely used of all drugs.

All the activators shared the property that they could generate NO and relax smooth muscle. In 1979, Louis Ignarro at Tulane University, New Orleans, found that bubbling NO near an isolated artery triggered a relaxation response. "I asked Murad: 'Why do our bodies have the machinery to respond to nitroglycerin?'," says Ignarro. "The obvious conclusion was that our bodies have an endogenous nitrogenous compound of some kind." But evidence for the endogenous compound was lacking and NO itself was not a good candidate. "Edwin Krebs said to me: 'It's a pollutant; it's a chemical in the air — why are you working on this?'," says Ignarro.

More puzzling information came from Robert Furchgott of the State University of New York (SUNY) in Brooklyn. He had been struggling

with the finding that acetylcholine, a known relaxant in the body, caused isolated blood vessels to contract. He returned to this quandary by mistake in 1978, when a technician left out a planned washing step in an unrelated experiment. For the first time the technician observed relaxation after *in vitro* application of acetylcholine.

The difference between the earlier and later experiments was in the method of preparing the blood vessels. In the earlier experiments Furchgott had made strips. But in carefully drawing the cut strips over his finger he had wiped off a critical layer of endothelial cells. The rings of vessels used in the later experiments retained the endothelial cells, so they could respond to acetylcholine to produce what Furchgott now dubbed endothelium-derived relaxing factor (EDRF).

From Furchgott's paper in 1980 it took six years to deduce that EDRF was NO. "Reading over these papers now it is amazing that we didn't draw these conclusions five years before," says Ignarro. But, he says, "very few people went out on a limb to suggest what EDRF might be." NO was implicated by evidence from researchers including Murad and Ignarro, but many feel that the knockout punch came from Salvador Moncada (then at the Wellcome Research Laboratories in Beckenham, England), who demonstrated in a 1987 *Nature* paper that the amount of NO produced by a known relaxant was sufficient to cause a full relaxant response. Unfortunately the Nobel Prize can be awarded to at most three people, so in a controversial decision the 1998 Nobel for Physiology or Medicine was presented to Murad, Ignarro and Furchgott.

NO does it all

As NO research boomed, scientists realized that NO not only relaxed blood vessels, but also acted as a messenger in the immune and nervous systems. This increased the number of possible applications of NO-related drugs, but also made it

more likely that there would be problems with side-effects. A number of inhibitors of NO synthases (NOS) fell into this trap: although they inhibited the immune system's inducible NOS (iNOS), they also jammed up the endothelial NOS (eNOS) and caused an increase in blood pressure (hypertension).

John Wallace (University of Calgary, Alberta, Canada) and Giuseppe Cirino (University of Naples, Italy) were interested in more subtle modifications of NO levels. It was well known that the beneficial effects of NSAIDs (including the painkillers ibuprofen, naproxen and aspirin) were compromised by their toxicity in the gastrointestinal (GI) system. "We identified that two of the key problems with NSAID GI toxicity were reduced blood flow and increased neutrophil adhesion," says Wallace. "Those were both things that we knew could be counteracted by NO."

This idea was the basis for NicOx. The NO is supplied by a nitroxybutylester moiety on the NSAID, which was designed and synthesized by Piero Del Soldato, now the executive vice president of research at NicOx. Cellular esterases release the parent NSAID, and cytochrome oxidases transform the nitrate moiety into NO.

Initial tests in animals and then humans brought a pleasant surprise. "We were very impressed that there was no modification in blood pressure," says Del Soldato. "This was unexpected." The key, he says, is the slow release of NO. "You are producing the NO at a level that is unable to overcome the homeostatic process. You release NO but in a controlled way."

The beneficial effects of the NO moiety extend beyond the prevention of GI ulceration and bleeding. Inflammation, says Del Soldato, "is already activated when you give the compound, so inhibiting one receptor is not enough." Luckily NO affects up to 7 or 8 different cell types involved in inflammation.

A virtual company

The NO-NSAIDs' main competition is the Cox2 inhibitors, which hit the inflammation-induced Cox2 enzyme, but spare its constitutive relative, Cox1. The selectivity is important because Cox1 helps to protect the stomach lining.

The Cox2 compounds nearly killed the NO-NSAID project before it started. Armed with an idea and a compound, Wallace says he and his colleagues "shopped it around to big pharma, [but] at the time these companies were developing Cox2 inhibitors so we didn't get much interest. We realized we would be better off developing it ourselves."

That was easier said than done. "For three years we lived on our own financial resources," says NicOx CEO Michele Garufi. "In Italy in 1994 it was impossible to find venture capital," and British venture capital "didn't invest beyond the Channel." Finally the money arrived but with one condition: that the company move to France.

Not that the team was forgotten in Italy. "We are very famous in Italy only because we are the first two crazy guys to do something that is quite normal in the US," says Garufi. "We were non-conventional Italians — we wanted to take risks."

The funding was still modest, so to make it last longer the company contracted most of its work out to university researchers. "We were a little forced to do that because we didn't have the funds to do otherwise," says Garufi. "But sometimes you are forced into a model and then you realize it's the right model." NicOx has stayed with contract research and even now has only 20 full-time employees.

Working in a field as vast as NO biology, Garufi says that "it would have been too pretentious and too limited to group within our company only a few good scientists. It's much more productive to have all the top professors working for us in their own laboratories or institutes."

"That gives you flexibility in closing your projects by finishing the

research contract rather than having to fire five people," he says. "And it gives a lot of credibility to our research. The data are coming from top level professors who have credibility and a reputation."

The main focus in-house is the chemical laboratories, which help the company keep the most important preclinical information proprietary. But if the company ever wants some biological work done "we always find more people than we need," says Garufi, "because NO is a hot field."

The number of collaborations has allowed the company to branch out. By adding NO-generating moieties to a variety of common drugs, the company is moving into areas such as urinary incontinence (both NO and NSAIDs have an effect), osteoporosis (NO inhibits bone-eating osteoclasts), asthma (NO and a steroid relax bronchial smooth muscles), thrombosis (NO augments the anti-clotting effects of aspirin), high blood pressure (NO may improve standard drugs such as beta blockers) and even Alzheimer's disease (with NO acting as an anti-inflammatory).

Even in pain and inflammation, Del Soldato feels NicOx has the upper hand. "In terms of safety we have an advantage because Cox2-specific inhibitors are good [only] when the tissue is not [already] compromised," he says. And, he says, "Cox1 is critical. It has a relevant role in inflammation and thrombosis." A recent study, for example, indicates that the heart-protecting effect of naproxen is lost when Merck's Cox2 inhibitor Vioxx is used instead.

"I saw the flaws in the Cox2 approach early on," says Wallace. "The marketing was getting way ahead of the science. We are being vindicated now." Garufi's assessment of the situation is more understated, perhaps more European. When NicOx began, he says, "there were many risks. But so far so good."

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