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EDITORIAL

Pilot studies: one swallow does not make a summer ...

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ABSTRACT

What should we expect from pilot studies, done in small series of patients? In the literature there are many examples of small studies with very promising results, that in subsequent larger or better controlled studies proved to be much less promising, or even disastrous. In some instances the initial favourable outcome was due to selection bias. In others the use of nonvalidated methods of measuring outcome made the reproducibility of promising observations problematic. However, we have to start somewhere. In ths issue The *et al*. report favourable results of granisetron treatment in four out of five patients with chronic fatigue syndrome. A prospective, randomised, placebo-controlled, double-blind clinical trial with granisetron in patients with chronic fatigue syndrome is now ongoing.

In this issue of the Netherlands Journal of Medicine, The *et al*. report a pilot study of granisetron treatment in patients with the chronic fatigue syndrome.[1] They treated five patients, all meeting the CDC criteria for chronic fatigue syndrome, with a four-week course of granisetron in an uncontrolled study. Using validated assessment instruments measuring fatigue severity and functional impairment, they found improvements in four out of five patients. Although due to the design of the study a placebo effect can not be excluded, the authors state that they have not encountered similar remarkable changes in fatigue severity and functional impairment in the placebo groups of previously performed placebo-controlled studies. The results of this pilot study have made them initiate a prospective, randomised, placebo-controlled, double-blind clinical trial with granisetron in patients with the chronic fatigue syndrome.

What should we expect from drugs evaluated in studies such as the one by The and colleagues? Treatment of patients with the chronic fatigue syndrome is not an easy task. Prescribing a drug is a lot easier than interventions such as cognitive behaviour therapy or graded exercise therapy. Although we would all be very happy with an effective drug therapy for chronic fatigue syndrome, the size of this pilot study does not permit high expectations. In the literature there are too many examples of studies with very promising results, which in subsequent larger or better controlled studies proved to be much less promising or even disastrous.

A first example is the use of nitric oxide synthase (NOS) inhibitors in the treatment of septic shock. Vascular endothelial cells make nitric oxide. This endothelium-derived nitric oxide stimulates cyclic guanosine monophosphate synthesis in the underlying vascular smooth muscle, causing relaxation. Overproduction of nitric oxide can lead to inappropriate vasodilation, loss of systemic vascular resistance and hypotension. In septic shock, such an overproduction of nitric oxide is attributable to a distinct, high output, cytokine- and endotoxin-inducible NOS isoform (iNOS) present in endothelial and/or smooth muscle cells.[2] Following encouraging studies in experimental settings clinical trials investigating the effects of treatment with an NOS inhibitor, N^G-methyl-L-arginine (L-NMMA), were initiated. In 1999, Grover *et al*. published the results of a multicentre, dose-ranging, safety study of 546C88 (NMA hydrochloride) for the treatment of septic shock

(n=32). This compound proved to be a potent vasoactive agent capable of restoring systemic vascular resistance, reducing or eliminating the need for concurrent epinephrine therapy.[3] A subsequent placebo-controlled, multicentre phase III trial was terminated after inclusion of almost 800 patients, when a safety analysis found significantly worse survival (p<0.005) among patients receiving 546C88. The adverse outcome can possibly be explained by the fact that L-NMMA not only inhibits the inducible NOS, but also constitutive isoforms of NOS. The primarily cardiac serious adverse events may have been due to direct cardiac toxicity of L-NMMA.[4] Development of NOS isoform-selective inhibitors may produce agents with a larger therapeutic index. A promising new treatment modality came to an early end.

Another example of a study with disappointing results is the ELITE II study.[5] The ELITE II study was the successor of ELITE I.[6] In ELITE I elderly patients (n=722) with symptomatic heart failure (NYHA class II-IV) were treated (double-blind) with losartan titrated to 50 mg once daily, or to 50 mg of captopril three times daily, for 48 weeks. An unexpected 46% lowering of mortality (a secondary endpoint) was observed with losartan compared with captopril (losartan 17 (4.8%) *vs* captopril 32 (8.7) deaths; risk reduction 46% (95% CI: 5-69%); p=0.035). In addition, losartan reduced the rate of all-cause hospital admissions, and was better tolerated than captopril, despite a similar persistent rise in serum creatinine concentrations (primary endpoint of the study). The apparent superior effects seen with losartan on morbidity and mortality were based on a small number of events that were not the primary endpoint. Therefore, a much larger, randomised double-blind trial, ELITE II, was designed to compare the effects of losartan with those of captopril on mortality, morbidity, safety and tolerability. In ELITE II, 3152 patients aged 60 years or older with heart failure (NYHA class II-IV) and ejection fraction <40%, were treated (double-blind) with losartan titrated to 50 mg once daily, or to 50 mg of captopril three times daily. Disappointingly, in this study losartan did not prove to be superior to captopril in improving survival. Mortality and sudden death did not differ significantly between groups. ELITE II did confirm the superior tolerability of losartan observed in ELITE I.

A third example of gradually decreasing enthusiasm is the story of recombinant activated protein C (rhAPC). In March 2001 investigators reported the results of a phase III trial enrolling 1690 patients with severe sepsis showing that rhAPC significantly reduced the absolute risks of death from 30.8% in the placebo group to 24.7% in the treatment group.[7] The prevalence of bleeding as a serious adverse event during the 28-day follow-up period was greater with rhAPC than placebo (3.5% *vs* 2.0%), but this difference did not reach statistical significance (p=0.06). In light of the high mortality with sepsis and lack of alternative therapies, these encouraging results were welcomed by many healthcare professionals who expected rhAPC to quickly become available for clinical use. However, the Food and Drug Administration (FDA) asked for additional phase III testing before a final decision about its use clinically was taken. The FDA, because of concerns, restricted its use to those with a high risk of death.[8] Further analysis of the phase III trial results showed that rhAPC was substantially more beneficial in the second than first half of the trial. This change in effect was associated with an amendment modifying trial enrolment criteria, and a change in the manufacturing of rhAPC.[9] Additional safety concerns were the high prevalence of serious intracranial haemorrhages reported in a compassionate use protocol, and the seemingly smaller efficacy in a less ill subpopulation. A similar relationship between risk of death and effect of treatment was also found for other mediator-specific anti-inflammatory agents.[10] Studies in septic patients with mild disease are underway. So far, rhAPC use is limited to severe sepsis only.

Fortunately not all initial good experience leads to disappointment later on. There are also many examples of promising small or uncontrolled studies that did turn out to become accepted therapies, on the basis of subsequent controlled clinical trials. Especially in the treatment of the acquired immunodeficiency syndrome AIDS, the early studies were mostly uncontrolled. Multiple randomised trials have led to the availability of 15 registered antiretroviral drugs in the Netherlands, which are now often used in combination therapy.[11]

Methotrexate is currently the most frequent choice of disease-modifying antirheumatic therapy for rheumatoid arthritis.[12] In the 1980s methotrexate was mostly used in individuals who had severe rheumatoid arthritis. Patients with more severe disease have a higher risk of cardiovascular death. In studies without adjustment for this confounding factor methotrexate use is linked to poor outcome.[13] However, uncontrolled observational studies suggested effectiveness of methotrexate for rheumatoid arthritis.[14,15] In double-blind, randomised studies the improvement of methotrexate on mobility and systemic inflammation was confirmed.[16]

A third example of the development of a successful therapy is the use of infliximab for Crohn's disease. *In vitro* studies suggested that the production of tumour necrosis factor α (TNF-α) in the mucosa of patients with Crohn's disease is increased.[17] Similar findings were reported for the synovia of patients with rheumatoid arthritis. In patients with rheumatoid arthritis, treatment with antibodies against TNF-α were found to reduce signs and symptoms of this disease.[18] This stimulated the use of anti-TNF-α in patients with Crohn's disease. In that preliminary trial of only nine patients, a remission occurred after one infusion of the antibody in eight patients.[19] In a subsequent double-blind,

placebo-controlled trial in 108 patients with treatment-resistant Crohn's disease the efficacy of the antibody was confirmed.[20]

The above is to illustrate that one swallow does not make a summer. Initial promising data need confirmation in larger, well-designed clinical trials. Only then will we be able to fully benefit from adequately tested drugs. Especially in diseases with a highly variable clinical course, such as sepsis, small uncontrolled series may suffer from important selection bias. The examples of rhAPC and L-NMMA show how careful we should be with the interpretation of pilot studies in the treatment of sepsis. Another reason for wrongful optimism after pilot studies is the use of nonvalidated methods of measuring outcome. Objective and accepted measures of disease activity, such as disability index scores in rheumatoid arthritis, make the reproducibility of promising observations more likely. Nevertheless, we have to start somewhere. Hopefully the concept of granisetron therapy for chronic fatigue syndrome will prove to be highly effective and safe. The good news is that upregulated serotonin seems to play a pathophysiological role in the neurobiology of chronic fatigue syndrome. This means that granisetron, a serotonin antagonist, is a rational therapy. Also, the methods to assess the effect of interventions are well established. We look forward to the results of the prospective, randomised, placebo-controlled, double-blind clinical trial with granisetron in patients with the chronic fatigue syndrome.

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