Calcium antagonists in stroke
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General discussion
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In normal conditions in a human brain, calcium ions (Ca$^{++}$) function as membrane stabilisers, metabolic regulators, and second messengers. They can activate intracellular cell degradative enzymes such as lipases, proteases, and endonucleases. In 1974, these ions were also found to play an important role in ischaemic myocardial cell death. In experiments with cerebellar tissue, anoxia appeared to trigger a rapid translocation of calcium ions from extracellular to intracellular spaces.

In cerebral ischaemia, signalling functions are lost rapidly because all available energy is required to maintain cell integrity. Depending on the duration and intensity of ischaemia, irreversible cell damage will ensue. Massive influx of calcium ions plays an important role in the deleterious events finally leading to cell death. In the core of focal cerebral ischaemia the chemical reactions very rapidly lead to cell death, but in the area surrounding this core, the so-called ischaemic penumbra, cells remain viable for several hours.

The finding of this time window led to an intensive search for drugs which were able to interrupt or delay the reactions leading to cell death. After two decades of animal experiments and clinical studies with several promising agents, there is no neuroprotective therapy available for patients with an ischaemic stroke. The history of treatment with calcium antagonists acting on voltage sensitive calcium channels, especially nimodipine in ischaemic brain lesions, is described in this general discussion.

The first in vitro and in vivo animal experiments with nimodipine demonstrated a relaxing effect on cerebral vessels. In 1982, its possible influence on focal cerebral ischaemia was reported after experiments in which nimodipine was started before induction of ischaemia. In 1986, the first results were reported of animal experiments in which nimodipine was started after induction of focal ischaemia and in which effectiveness was assessed in comparison with control animals.

We systematically reviewed all controlled animal experiments with nimodipine in focal cerebral ischaemia (this thesis, chapter 6). We selected experiments in which treatment was started after the induction of ischaemia and if effects had been assessed in animals or in whole brains, not in slices or small samples of brain tissue. The results of the 20 included studies were inconsistent: Analysis yielded 10 positive and 10 negative studies (as concluded by the authors). In-depth analysis of data of infarct size and amount of oedema revealed a statistically significant effect in favour of nimodipine. However, these data were available from a small selection of studies, mainly studies in favour of nimodipine. The methodological quality of the animal experiment was assessed with a scoring system based on recommendations published in 1999. The quality of the animal experiments with nimodipine appeared to be poor, only 2 studies
mentioned randomisation of animals, in not more than 7 outcome was assessed by an assessor blinded for treatment allocation. Although 8 studies assessed two outcome measurements (functional and histopathological), only 3 studies assessed outcome in the chronic phase. Remarkably, in one study, according to the authors, double blind assessment of outcome had been performed.31

In 1982, the first results of clinical studies were reported by Gelmers who had studied the effect of nimodipine on the cerebral blood flow in 10 patients with acute ischaemic stroke.207 Based on the hypothesis of the calcium dependent final common pathway in cell death and his previous experiments, Gelmers started a single blind pilot study, which suggested that treatment with nimodipine was very promising.42 In 1988, the same author reported the results of a placebo controlled, double-blind, randomised clinical trial.43 In the introduction of the manuscript various results of animal experiments with nimodipine were quoted, one experiment in focal cerebral ischaemia with beneficial results,28 and experiments with global ischaemia, cerebrovascular damage in spontaneously hypertensive stroke prone rats, or the use of nimodipine before induction of ischaemia. The authors of the report on the randomised clinical trial concluded that, 4 weeks after stroke, nimodipine was statistically significantly better than placebo with regard to mortality and changes in neurological deficit (assessed with modified Mathew scale). However, re-analysis of data on the functional outcome of patients in this trial, by dichotomising the score on the disability item in the modified Mathew scale, shows a difference which was not statistically significant.123 (This thesis, chapter 4)

The study with the supposedly positive results of Gelmers et al. was followed by a small trial by Paci et al,107 who had similar results. Subsequent reports on larger clinical trials44-46,113 refer in the introduction to a single positive animal experiment, to the proven effectiveness of treatment with nimodipine in subarachnoid haemorrhage, but primarily to the study of Gelmers et al. Reports on animal experiments with nimodipine, positive and negative, continued to appear in the literature until 1995. It is surprising that these animal experiments ran a course parallel to several clinical studies, since we would expect that the clinical studies are preceded by animal studies.

The supposedly positive results of the first clinical studies with nimodipine could not be confirmed in subsequent large trials. Other studies with calcium antagonists acting on voltage sensitive calcium channels, such as flunarizine and isradipine, also failed to show beneficial results.51,54 A post-hoc subgroup analyses on data of patients treated within 18 hours after onset of symptoms in the American nimodipine trial, showed that this subgroup had a significantly lower number of patients worsening on day 4 and an improvement of the average neurological scores during treatment.46 The final outcome in this subgroup was not reported. This finding was used to support the idea that clinical studies failed to demonstrate beneficial effects, because
treatment had been started too late (patients were included up to 48 hours after stroke onset). This hypothesis was further investigated in a meta-analysis of trials with nimodipine.\textsuperscript{47} No effect of nimodipine was found in the overall analysis, but in the subgroup analysis of patients treated within 12 hours of stroke onset, the odds ratio for neurological impairment was 0.62 (95\% C.I. 0.44 to 0.87) in favour of nimodipine. Similar results were reported for functional outcome assessments. Nimodipine started 12 to 24 hours after stroke onset did not change outcome, treatment started later than 24 hours significantly worsened outcome.

The VENUS study was started to test the hypothesis that early treatment with nimodipine is beneficial. As described in this thesis (chapter 2), the study was terminated early, when in our Cochrane review the beneficial effects of neither early treatment with calcium antagonists in general, nor with nimodipine in particular could be confirmed.\textsuperscript{123}(This thesis, chapter 4)

Some remarks can be made about the methods used in the meta-analysis on which the VENUS study was based.\textsuperscript{47} The authors decided not to include unpublished trials, more specifically, they only included trials of which data were available in the Bayer AG database. This led to exclusion of two negative studies which were performed by independent trialists\textsuperscript{115,159} and to exclusion of a small pilot study.\textsuperscript{106} Exclusion of unpublished trials, either because they were not identified or were deliberately excluded, have been shown to lead to misleading results,\textsuperscript{170,171} and usually to exaggerated estimation of intervention effectiveness.\textsuperscript{215}

The statistical analyses of the meta-analysis, on which the VENUS study was based, were performed by a statistician of a pharmaceutical company, after the authors of the manuscript had decided which outcome measures (which cut-off points) had to be used and which subgroup analyses had to be performed. The “intention-to-treat” principle was applied in the analyses, but it remained unclear how missing values were handled. These missing values must have been extensive, as in the graphs presenting the results, different numbers appeared in the denominator of each analysis. In the final manuscript, numbers of patients with poor outcome in each included trial were lacking, which made direct comparison of this meta-analysis with our systematic review impossible. Both the authors and Bayer AG were unable to provide these data.

In retrospect, we can conclude that on the first waves of positive news about the beneficial effect of calcium antagonists in the treatment of cerebral ischaemia, the subsequent steps in research have gone too fast. The few available data from animal experiments were not addressed critically, which too early led to clinical studies. Remarkably, animal experiments continued to be started, while several clinical studies had already been performed or were in progress. In order to prevent similar mistakes in the future, adherence to guidelines for pre-clinical experiments, which have recently been published, may be helpful.\textsuperscript{180} Animal experiments should define an optimum dose and time window for treatment, as well as an optimal duration of
treatment. Effectiveness should be investigated in randomised groups of both small and larger animals, and outcome (both histological and functional in acute and chronic phase) should be assessed by a blinded assessor. In these experiments, physiological parameters have to be monitored. After the results of such animal studies appear to be favourable, it is time to start clinical studies. Guidelines for manuscripts presenting results of clinical trials and systematic reviews should enable interpretation of the data.216,217

By going back in time it was possible to reconstruct the steps that led to the inclusion of more than 7500 patients in studies with calcium antagonists in acute stroke, a treatment which finally turned out to have no effect on outcome. Enthusiastic researchers sometimes seem to lose their critical attitude, and start and continue clinical studies based on too weak scientific evidence.