

COSBID

*Cooperative Studies On Brain
Injury Depolarisation*

17TH ANNUAL MEETING, **COSBID 2015**

September 9th - 11th



EXHIBITORS



Welcome

*It is a great pleasure to welcome you to the 17th COSBID meeting
in the beautiful setting of Helsingør (Elsinore).*

Almost 12 years have passed since the original meeting in Copenhagen in December 2003, and much has been achieved since then on discovering the clinical importance of spreading depression and brain injury depolarizations. The gradual incooperation of basic CSD research into the meetings is an important development that may pave the road to achieving an efficient treatment paradigm to antagonize secondary brain injury. The clear overweight of basic papers in the present program speaks for itself.

The scientific committee has shown excellent collaboration in framing the program: Jens Dreier, Cenk Ayata, Raimund Helbok, Martin Lauritzen and Martin Fabricius. Jed Hartings continuing efforts keeping the homepage updated are highly appreciated.

We gratefully acknowledge our exhibitors: Moberg ICU Solutions, Ad-Tech Medical Instruments Corp., and Cephalon. Without their generous support, this program would not be possible.

Finally we thank the staff at Konventum for all their help and patience shaping the backbone of the meeting.

We hope you will enjoy your stay at the Konventum, which contains the largest private collection of art in Denmark. The paintings of our foremost modern artists are present everywhere. We also hope weather will allow you to take a stroll in the park, and by all means, do not miss a visit to Hamlets castle, Kronborg.

Have a great stay!

Martin Lauritzen & Martin Fabricius

Programme

September 9th - 11th 2015

WEDNESDAY, SEPTEMBER 9TH

17.00-18.00 Arrival, registration

18.00-18.05 Welcome. *Martin Fabricius & Martin Lauritzen.*

18.05-18.50 Invited evening talk

Chair: Martin Lauritzen

Maiken Nedergaard: Glymphatic system in health and disease

19.00-19.30 Pre-release viewing of documentary video "COSBID"

19.30-21.30 Get together - light fare and wine



THURSDAY, SEPTEMBER 10TH | SCIENTIFIC SESSIONS

8:00-09:40 TBI models

Chairs: Anthony Strong and Jed Hartings

08:00-08:12 Cortical Depolarization Associated with Brain Retraction Injury.
Michael Ayad

01

08:20-08:32 Real time glutamate dynamics during cortical spreading depolarizations.
Baptiste Balanca, Thomas Lieutaud, Anne Meiller, Stéphane Marinesco

02

08:40-08:52 Non-propagating, multi-focal mini acute depolarisations of the injured human brain: Artefact or biological?
Sharon Jewell, Jordi Serra, Asma S Khan, Martyn Boutelle, Clemens Pahl, Anthony J Stong

03

09:00-09:15 Spreading Depolarizations in a Swine Model of Traumatic Brain Injury.
Jonathan York, Jason M. Hinzman, Eric Mahoney, Jed A. Hartings

04

THURSDAY, SEPTEMBER 10TH | SCIENTIFIC SESSIONS

09:40-10:10

Coffee break & exhibitions



10:10-11:50

Hypoxia and ischemia models

Chairs: Jens Dreier and Eszter Farkas

10:10-10:22

The impact of age on the evolution of spreading depolarization triggered during global forebrain ischemia in the rat

Ákos Menyhárt, Péter Hertelendy, Péter Makra, Borbála Szepes, Orsolya M. Tóth, Ferenc Bari, Eszter Farkas.

05

10:29-10:41

Fifteen to 20 minutes of anoxic spreading depolarization (SD) in the brain cortex are fully reversible after middle cerebral artery occlusion (MCAO) in rats.

Janos Lückl, Uldus Khojasteh, Ana I. Oliveira-Ferreira, Sebastian Major, Jens P. Dreier.

06

10:48-11:03

Hemodynamic Polymorphism of Normoxic and Hypoxic Spreading Depolarizations in Clusters in the Gyrencephalic Swine Brain.

Renán Sánchez-Porras, Michael Schöll, Edgar Santos, Humberto Silos, Yuan Gang, Modar Kentar, Daniel Hertle, Andreas W. Unterberg, Oliver W. Sakowitz

07

11:12-11:27

Is focal ischemia necessary for the occurrence of spontaneous spreading depolarizations after subarachnoid hemorrhage?

Fumiaki Oka, Shin-Pin Chen, Ulrike Hoffmann, Jeong-Hyun Lee, Kristen Park Hopson, Izumi Yuzawa, Tao Qin, Cenk Ayata

08

11:50-13:00

Lunch



13:00-14:45

Basic mechanisms and Optical imaging I

Chairs: Cenk Ayata and Kevin Brenman

13:00-13:10

Effects of Magnesium on spreading depolarizations in gyrencephalic brains.

Edgar Santos, Fiorella León, Humberto Silos, Renan Sanchez-Porras, Andreas Unterberg, Oliver W. Sakowitz

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THURSDAY, SEPTEMBER 10TH | SCIENTIFIC SESSIONS

13:00-14:45

Basic mechanisms and Optical imaging I

Chairs: Cenk Ayata and Kevin Brennan

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Chair: Martin Fabricius

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Cortical Depolarization Associated with Brain Retraction Injury

Michael Ayad, M.D., Ph.D.

OBJECTIVE

Based on the work of Meyer & Denny-Brown in 1955 and others, it is reasonable to suspect that an “injury potential” might be associated with damage to the brain from excessive retraction, e.g. during skull base operations. To better understand its pathophysiology, this study investigated the correlation between brain retraction pressure (BRP), local cortical DC potential and histological injury resulting from controlled temporal lobe retraction in rabbits.

METHODS

Retraction of the temporal lobe for either 15 or 30 min was carried out in halothane/nitrous oxide anesthetized New Zealand white rabbits at in initial BRP of 20, 30 or 40 mm Hg (n=5 each of 6 groups, sham n=5). Using a specially designed fluid-filled sensor containing platinum electrodes and rigid head fixation, BRP, cortical DC potential and 4 channels of ECoG were continuously monitored on a digital polysomnograph. After 8 hrs of reperfusion, the brains were removed, cut in 2 mm coronal sections, stained with 2,3-TTC and formalin-fixed for histological analysis by a neuropathologist, blinded to the experimental group.

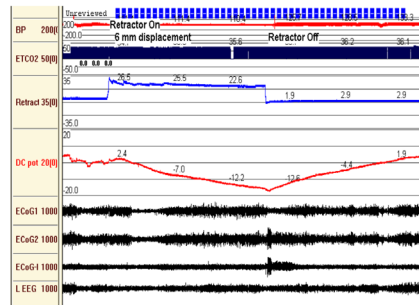
RESULTS

The brain retraction pressure-time index, calculated as the area under the retraction pressure curve, was found to correlate in a highly significant manner with both the integral of the DC potential shift observed, as well as with the volume of injury determined by morphometric analysis ($p<.0001$ for both, $r=0.79$ and $r=0.90$ respectively). In addition, the DC potential integral was significantly correlated with volume of injury ($r=0.91$). Using multivariate regression, pressure was found to have approximately one and one half times the rela-

tive contribution of retraction-duration to the volume of injury.

CONCLUSIONS

Although spreading of activity did not occur, cortical depolarization associated with brain retraction bears resemblance to that seen with other types of injury. Excitotoxic mechanisms appear to be involved from ischemia and local trauma. The pathophysiology of injury is a function of both the magnitude as well as the duration of retraction.



Real time glutamate dynamics during cortical spreading depolarizations

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INTRODUCTION

Cortical spreading depolarization (CSD) are thought to worsen brain damage after severe acute brain injury. N-methyl-D-aspartate receptors (NMDAR) seems critical for CSD propagation, as CSD can be suppressed by its antagonists and evoked by its agonists. Therefore, based on indirect argument, excitotoxicity is believed to play an important role during CSDs. However glutamate extracellular concentration's (GEC) modifications during CSD remain unknown. In our study, we intend to describe real time GEC dynamics during isolated and clusters of CSDs in vivo, after brain trauma.

METHODS

Wistar rats were anesthetized with isoflurane in a mixed air - oxygen (30%), intubated and artificially ventilated prior brain trauma. Isolated CSD were either triggered by KCl apposition on a normal cortex (KCl group; n=6) or occurred spontaneously following a severe (3.8 ATA) lateral fluid percussion brain injury (LFPI group, n=6). Finally clusters of CSDs (i.e. 3-5min CSD interval) were triggered by KCl apposition after LFPI (Cluster group, n=6). We used a 1 mm laser Doppler flowmeter (LDF) probe for cerebral blood flow, and a glass micropipette for local field potentials recordings. GEC was measured second by second using microelectrode biosensors (40 μ m tip) consisting in a platinum wire inserted in a glass micropipette and covered with a specific oxidase. Analyses were performed in a 5min window flanking CSDs. Results are expressed as median and interquartile range.

RESULTS

CSD were associated with a hyperemic re-

sponse in both group, the area under the curve of the LDF response being lower after LFPI (4957 [2861-6260] %BPU.sec) than in the KCl group (22480 [19140-29830] %BPU.sec, $p < 0.01$). Overall GEC peak occurred 18 [8 - 40] sec before the LDF one, and 14 [-11 - 25] sec after the depolarization's nadir. There was a non-significant trend for glutamate release to be larger after LFPI (667 [315-1372] μ M.sec) than in the KCl group (139 [20-459] μ M.sec, $p = 0.17$). GEC prior and after CSD remain below 1 μ M. Interestingly as CSD repeat within a cluster, glutamate release decrease significantly from 22.2 [20.5-25.7] μ M to 3.9 [3.5-5.7] μ M after 10 CSDs ($p < 0.001$), without basal GEC increase.

CONCLUSION

The present study describes real time GEC dynamics in vivo after CSDs in a normal and traumatized cortex. We evidence a transient glutamate increase preceding LDF response. The magnitude of this release seems not to be affected by brain aggression. Overall, our data confirm the central role of glutamate. However if CSD occurs too close to each other glutamate release almost disappears, suggesting a delayed pre-synaptic vesicles re-filling processes.

Non-propagating, multi-focal mini acute depolarisations of the injured human brain: Artefact or biological?

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Clemens Pahl(4) Anthony J Strong(1).

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BACKGROUND

It is well established that propagating waves of prolonged neuronal/astroglial depolarisation correlate with delayed neurological deficit, expanding infarct size and poor outcome following haemorrhagic and traumatic brain injury. The electrophysiological characteristics of spreading depolarisations (SD) have been well described and in AC-coupled recordings typically present with a large (0.5–4mV) poly-phasic slow potential change that creeps across the cortex at a rate of 2–7mm/min and is customarily accompanied by a transient depression of background rhythms. There are however, still ECoG phenomena yet to be fully explained.

Objective: To describe our observation of small but stereotyped slow negative potential inflections which lack the hallmarks of classical SD but are striking in some subjects and do not appear clearly artefactual.

METHODS

Six patients with acute brain injury were monitored for SD with simultaneous subdural strip (6 platinum contacts, exposed Ø2.3mm, 10mm centre-to-centre) and intraparenchymal depth (8 platinum contacts, Ø1.1mm, 1.3mm long, 2.2mm centre-to-centre, Adtech, USA) electrodes referenced to the scalp. Signals were high-pass filtered at 0.02Hz, digitized at 1kHz and recorded using Labchart (ADInstruments, Australia). Recording times ranged from 25–110hrs.

RESULTS

In 4/6 subjects, classical SDs appeared both on subdural and intraparenchymal electrodes. The analysis was performed on the strip re-

cordings only. In 5/6 patients, we observed stereotyped slow negative variations in the baseline that reached a maximum amplitude of 560µV over the course 4–10s and a return to baseline over a subsequent course of 8–30s. The events occurred independently from the classical SDs. A widespread multi-focal distribution across all contacts was observed in some subjects whilst in others, events were restricted to a limited number of contacts. The events showed no propagation and there was no ensuing depression. In one subject, electrical seizures were observed riding the waveform. The events, which were not associated with movements or physiological deregulations, occurred in varying number, ranging from 2 to >1000/24h.

DISCUSSION

In addition to classical SDs, there are stereotyped slow negative shifts in the baseline (...) that may represent mini acute depolarisations (MAD) of areas surrounding a site of primary injury. Events appear to be insufficient in duration and/or are restricted to a cell population too small to induce identifiable silencing of background rhythms. We submit this material for discussion of the basis of the findings.

CONCLUSION

If proved not to be artefactual, MADs may hold important clinical significance in the context of human brain injury.

SUPPORT

Supported by Wellcome Trust Department of Health (England and Wales) Health innovations Challenge Fund (Dept Health Ref: HICF-1010-080; Wellcome Trust Ref: WT094912)

Spreading Depolarizations in a Swine Model of Traumatic Brain Injury

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INTRODUCTION

Clinical studies of severe traumatic brain injury (TBI) have shown that spreading depolarizations (SD) are an important mechanism of secondary injury. SDs occur variably in ~55% of patients and are independently associated with worse long-term outcomes. However, clinically relevant animal models to study the implications of SD for therapy and pathogenicity are lacking, as spontaneous SDs do not occur in commonly used rodent TBI models. The aim of this study was to develop a gyrencephalic TBI model to examine the role of SD in the development of traumatic contusions.

METHODS

In 80–100 lb Yorkshire swine, anesthesia was induced with telazol/xylazine and maintained with propofol. Animals were intubated and ventilated with room air, and arterial pressure, EKG, and SpO₂ were monitored. To induce TBI, a 22 mm-diameter burr hole was drilled over fronto-parietal cortex and a 100 gm impactor rod (15 mm diameter) was dropped from 80 cm onto the exposed dura through an open lumen tube. Two subdural electrode strips were then positioned to span from the impact site in anterior, posterior, and lateral directions, and animals were monitored for 6–7 hours before euthanization. Post-mortem brain MRI scans were obtained using T₂ FSE and GRE sequences. The brain was then harvested, sliced, and stained with 2,3,5-triphenyltetrazolium hydrochloride (TTC).

RESULTS

In 6 animals, TBI resulted in substantial but variable cerebral contusions as judged by T₂ signal alteration, with lesion volumes ranging

3.4 ml to 9.3 ml (9 to 24% of hemispheric volume). Similarly, hemorrhage was often severe but variably present in different intracranial spaces. SDs arose spontaneously in 83% (5/6) of animals, beginning 30–60 min after TBI and occurring throughout the monitoring period. Total SD counts ranged from 1 to 15. An additional 19 animals were studied in a model development phase, using different anesthesia (isoflurane), ventilation with 100% O₂, and variable heights of impactor drop. SDs were observed in 73.6% (14/19) of these animals and T₂ lesion volumes ranged 1.19 to 8.82 ml in 15 of 19; 4 had no lesions. Overall, animals with no lesions on TTC staining were less likely to exhibit SD (2/5, 40%) than those with TTC lesions (17/19, 89%) (Fisher's, $p=0.04$), but lesion size did not correlate with SD number ($p=0.43$).

DISCUSSION

This is the first animal model of TBI to exhibit repetitive SDs as a common pathophysiologic mechanism, replicating a key aspect of TBI in humans. Substantial lesion volumes and the combination and variability of contusional and hemorrhagic components further establish the model's clinical relevance. However, less variability in pathoanatomy and SD activity would be needed to test therapeutic interventions.

The impact of age on the evaluation of spreading depolarization triggered during global forebrain ischemia in the rat

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OBJECTIVES

Spreading depolarizations (SD) spontaneously occur in the ischemic brain, and facilitate cell death. Aging is the most important risk factor for the incidence of ischemic stroke; yet, it is not clear how age alters SD evolution. The aim of the recent study was (i) to compare the typical features of K⁺-evoked SDs during cerebral ischemia in young and aged rats and (ii) to assess the impact of age and ischemia on the electrical threshold of SD elicitation.

METHODS

Adult male Sprague-Dawley rats of various ages were anesthetized with isoflurane in N₂O/O₂. Transient global forebrain ischemia was induced by bilateral occlusion of the common carotid arteries (2VO, in young adult and old rats, from 7-week-old to 2-year-old, n=52). Sham-operated rats (SHAM) served as control (n=11). DC potential and local cerebral blood flow (CBF) were acquired via a small craniotomy. SDs were elicited by topical application of 1 or 3M KCl through a second craniotomy (8-week-old group, n=14; 2-year-old group, n=11), or by electrical stimulation for the determination of SD elicitation threshold (7-30-week-old group, n=38). In the latter case, 3 SDs were evoked during baseline and another 3 SDs during ischemia in each experiment. Blood pressure was continuously monitored and blood gases regularly controlled via a femoral artery catheter.

RESULTS

Ischemia elongated the duration of the SD-related negative DC shift, which was augmented further by age. Old age was also associated with reduced slope of depolarization and amplitude

of repolarization. In animals 7-30 weeks old, brain maturation markedly increased the elicitation threshold of SD, and ischemia significantly elevated the threshold of SD elicitation. CBF decreased progressively during ischemia in the 2-year-old animals, and inverse neurovascular coupling with SD evolved in the old but not in the young ischemic groups. The hyperemic CBF responses to SDs were of smaller amplitude but longer duration under ischemia.

CONCLUSIONS

We propose that (mal)adaptation of cerebrovascular function with aging impairs the SD-related CBF response. As a consequence, the aged ischemic brain has scarcer resources to recover from an SD event, which reflects the increased vulnerability of the aged brain to ischemia- and/or SD-related injury. Finally, the threshold to trigger SDs increases with aging and ischemia. The susceptibility of the brain to SD may be determined by the gray matter's biochemistry and cytoarchitecture that undergo adaptational changes with maturation, and ischemia is suggested to inhibit SD elicitation by decreasing tissue pH.

FUNDING

Hungarian Scientific Research Fund (K111923), János Bolyai Research Scholarship of the Hungarian Academy of Sciences, Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-1/13.

Fifteen to 20 minutes of anoxic spreading depolarization (SD) in the brain cortex are fully reversible after middle cerebral artery occlusion (MCAO) in rats

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SD is the generic term for regenerative, “all-or-none” type depolarization waves in gray matter of the brain, characterized by abrupt, near-complete break-down of the neuronal transmembrane ion gradients with marked loss of Gibbs free energy. In this process, intracellular calcium rises to toxic levels. After MCAO, the first SD erupts after 1–5 minutes. After the first SD that originates in the ischemic center and concentrically invades the penumbra and surrounding tissue, subsequent SDs are generated at the rim of the permanently depolarized core and cause it to grow in stepwise fashion. SD in the center is often called anoxic SD. Despite important mechanistic differences between anoxic SD and SD in healthy surrounding tissue, Somjen listed a number of arguments suggesting that anoxic SD is not a separate entity but belongs to the SD continuum. Among these arguments is the fundamental feature that short-lasting anoxic SD is fully reversible in similar fashion to SD in healthy tissue, as studied in brain slices. Here, we investigated this in rats using filament occlusion of the MCA and infarct volume analysis after 72 hours, using coronal hematoxylin-eosin stained brain sections. In groups 1A (epidural electrocorticography (ECoG), n=28) and 1C (scalp electroencephalography (EEG), n=12), filament occlusion lasted for 90min, and in group 1B (epidural ECoG, n=8) for 15min. In the 2 groups with 90min of filament occlusion, 32 of 40 animals showed a cortical infarct (average size: $77 \pm 37 \text{ mm}^3$), and 36 of 40 a striatal

infarct (average size $40 \pm 17 \text{ mm}^3$).

Linear regression found that cortical and striatal infarct sizes significantly correlated (adjusted $R^2=0.537$, $P<0.001$, $n=40$). In the 15min-ischemia group 1B, no necrotic cells were found in either cortex or striatum. In conclusion, similar to SD in healthy tissue, anoxic SD is fully reversible in severely ischemic tissue when it does not outlast Somjen's commitment point.

Hemodynamic Polymorphism of Normoxic and Hypoxic Spreading Depolarizations in Clusters in the Gyrencephalic Swine Brain

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ABSTRACT

We investigated the spatiotemporal dynamics and hemodynamic patterns of normoxic and hypoxic spreading depolarizations (SDs) in clusters in the gyrencephalic swine brain. Normoxic depolarizations were triggered by KCl droplet stimulation, while hypoxia / ischemia was generated by permanent transorbital middle cerebral artery occlusion. On the cortical surface intrinsic optical signals (IOS) were recorded; additionally we validated the presence of depolarizations in some animals using electrocorticography (ECoG).

After KCl stimulation normoxic-SDs initiated as either concentric-radial (50%) or irregular-broken-radial (50%) waves that propagated in several semi-planar (100%) fronts following the gyri or across the sulci. Wave expansion morphology was determined by the cortex surface and anatomical structures. Repeated SD were mostly due to spontaneous SDs coming from the stimulation point, however, reverberating waves due to a continuous cycling around a gyrus were also observed. After clipping a drastic intensity change in IOS was seen in the cortex area supplied by the MCA. Hypoxic-SDs developed spontaneously from different sites of the hypoperfused cortex. Subsequent hypoxic-SDs continued to appear spontaneously from one side of the lesion. Hypoxic-SDs initiated mainly as irregular-broken-radial waves with semi-planar fronts of propagation following the gyri structure of the cortex. Deterioration of the hypoperfused cortex was observed

after the development of several hypoxic-SDs. Normoxic-SDs and hypoxic-SDs shared patterns of CBV changes. Nevertheless, differences in intensity and duration were found. Two main transitional patterns were distinguished: (1) a biphasic pattern with either an -oligemic or -hyperemic front, and (2) a triphasic pattern with mixed responses.

Our results indicate that anatomical structures contribute to the heterogeneity of spatiotemporal dynamics, while the hemodynamic reserve and ion diffusion to the continuum of regional hemodynamic polymorphism.

KEYWORDS

cluster; electrocorticography; ischemia; intrinsic optical signal imaging; spreading depolarization; stroke

Is focal ischemia necessary for the occurrence of spontaneous spreading depolarizations after subarachnoid hemorrhage?

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OBJECTIVES

Delayed cerebral ischemia (DCI) is a devastating complication of subarachnoid hemorrhage (SAH). Based on recent clinical and experimental data, cortical spreading depolarizations (CSDs) may cause DCI. Although the chemical milieu created by red blood cell lysis is thought to facilitate CSDs after SAH, the actual trigger mechanisms are unknown. We aimed to reproduce spontaneous CSDs in experimental SAH in rodents and explore facilitating and trigger mechanisms.

METHODS

Mice (C57BL/6, male, n=221) and rats (Sprague-Dawley, male, n=24) were used. SAH was achieved by injecting non-heparinized arterial blood (vs. saline) into the pre-chiasmatic cistern or the cisterna magna. Cortical electrophysiology, laser Doppler (LDF) or speckle flowmetry (LSF), and transient filament (fMCAO) or distal (dMCAO) middle cerebral artery occlusion outcome assessments were performed after SAH.

RESULTS

A total of 90 hours of cortical DC recordings using micropipettes between 3-9h, 12-18h and 72-78h time points after SAH in 15 mice failed to detect even a single spontaneous CSD, while an induced CSD was successfully detected in each case confirming technical integrity. Indeed, susceptibility to KCl- or electrical-induced CSD was suppressed 12h after SAH and normalized within 72h in mice; SAH never facilitated CSD susceptibility. The same reduction in CSD susceptibility was found 5d after

a double cisternal injection model of SAH in rats. Peri-infarct depolarization (PID) frequency after fMCAO was suppressed 12h after SAH, similar to KCl-induced CSDs. However, PIDs increased within 72h and normalized within 7d after SAH in mice. Transient fMCAO induced 12h or 72h after SAH caused 50% and 40% larger infarcts, respectively, compared with sham (non-SAH) controls. Perfusion defects were 50% and 70% larger during dMCAO induced 12h or 72h after SAH as shown by LSF in mice, suggesting primary vascular dysfunction.

CONCLUSION

Our data strongly suggest that elevated CSF K⁺ is by itself insufficient to trigger CSDs after SAH, although it facilitates PID occurrence if and when the tissue becomes ischemic.

Effects of Magnesium on spreading depolarizations in gyrencephalic brains

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INTRODUCTION

Blocking spreading depolarizations (SD) might improve outcome in brain-injured patients. Magnesium is known to be a neuroprotective, nevertheless, i.v. administration of Mg does not improve clinical outcome according to phase III studies. The aim of this study was to characterize the effects of Magnesium sulphate (MgSO_4), using i.v. bolus and local administration of MgSO_4 , on dynamics of blood volume changes using intrinsic optical signal imaging (IOS) and electrical characteristics using ECoG during the induction, and propagation of SDs in the porcine brain.

METHODS

Fifteen anaesthetized male German Landrace swine (mean weight of 29,3 Kg) were craniotomized and monitored over 6 hours. The groups were divided in control, I.V. Mg therapy or local Mg application. Video recording was implemented using a camera with an optical band-pass filter (564nm, FWHM: 15nm) and a light source. Blood volume changes in the cortex surface were visualized over time in both hemispheres. ECoG was measured using a subdural stripe electrode on the right hemisphere (Ad-Tech, USA). The brain was kept in a pool of paraffin. SDs were induced by hourly K⁺ stimulation (7mmol/l Ringer) in selected gyri of both hemispheres. After evocation of two SDs, the medium was washed out either with a physiologic solution and a solution containing 20mMol/l Mg in 0.9% NaCl for 10 minutes or either a 0.9% NaCl solution or NaCl 0.9% with 4g Mg was administered intravenously according to the group. One stimulation followed to

evaluate the effect of the therapy after 10 minutes and one more after an hour. $P < 0.05$ was considered statistically significant.

RESULTS

I.V. Mg therapy showed no significant effects on SD amplitude, velocity, area of expansion and duration using IOS or on amplitude and duration using ECoG compared to controls. Local application of Mg led to a complete inhibition of SDs detected through IOS and ECoG after ten minutes. One hour after washing out the topical Mg, a recurrence of the SDs in was appreciated in 50% of the cases. Those SDs had a decrease in amplitude, duration of oligemia total duration and velocity and reduced amplitude in ECoG.

CONCLUSIONS

Local Mg application but not i.v. application was effective to suppress SD blood volume changes measured by IOS and ECoG. Local Mg reduced the amplitude of depolarization measured by both ECoG and IOS and decreased durations of SD and velocity in IOS.

The neuronal endoplasmic reticulum undergoes rapid and reversible fission in vivo. The phenomenon correlates with changes in intracellular calcium and neuronal activity following cortical spreading depression.

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BACKGROUND

The neuronal endoplasmic reticulum (ER) is the major intracellular Ca^{2+} store that modulates synaptic transmission and plasticity. The ER structure is continuous throughout all neuronal compartments and permits the integration of spatially separated events, e.g. synaptic activity with nuclear responses. Here, we describe the hitherto unknown phenomenon of the rapid neuronal ER fission (=fragmentation) in living brain that results in transient loss of ER continuity. The ER fission-fusion correlates with intracellular Ca^{2+} rises and changes in neuronal electrophysiology.

METHODS

The ER was assessed with in vivo 2-photon microscopy in the cortex of transgenic mice expressing EGFP targeted to ER lumen. A custom-developed ER profile plot variance analysis and fluorescence recovery after photobleaching (FRAP) was used to quantify the state of ER continuity. NMDA receptor hyperstimulation, previously shown to alter ER structure in vitro, was induced using an experimental model of cortical spreading depression (CSD). Intracellular Ca^{2+} was monitored with the far-red Ca^{2+} indicator Asante Calcium Red. Simultaneously with imaging, we performed assessment of direct current (DC) and electrocorticography (ECoG). All experiments were approved by the Danish National Ethics committee.

RESULTS

The ER in its basal state appeared continuous with clear spine-ER morphology and the dendritic ER tree could be imaged up to 450 μm below the brain surface. With the onset of CSD, the ER underwent rapid (~10s) fission, assuming the form of numerous, seemingly isolated structures. With the recovery from DC shift, the ER regained its pre-CSD morphology within ~1 min, as assessed optically. The ER fission-fusion could be induced multiple times within the same neuron and coincided with rapid changes in DC. The phenomenon was a highly localized process and the ER fragmentation correlated with the rise in intracellular Ca^{2+} levels. Although ER appeared fused after the DC shift, the FRAP analysis revealed the existence of an ER fraction that remained isolated even ~2h after the onset of CSD. (...)

The increase and decline in the amount of non-continuous ER during CSD mirrored the loss and regain of neuronal activity measured with ECoG.

SUMMARY

We show that the neuronal ER can rapidly, reversibly and repeatedly alter its continuity in vivo. The phenomenon coincides with intracellular Ca^{2+} rises and the post-CSD regain of neuronal activity correlates with ER structural dynamics. It is of relevance since transient loss of ER lumen continuity may affect intracellular Ca^{2+} signaling, trafficking and homeostasis, and thus many vital aspects of neuronal function.

Susceptibility of primary sensory cortex to spreading depolarizations

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OBJECTIVE

Spreading depolarizations are increasingly recognized as important actors in neurological disease as diverse as migraine, stroke, subarachnoid hemorrhage, and brain trauma. Migraine aura involves sensory percepts, suggesting that primary sensory cortices might be intrinsically susceptible to spreading depolarizations.

METHODS

We used optical and magnetic resonance imaging, field potential and potassium electrode recordings in mice; and electrocorticographic recordings in humans, to determine the susceptibility of different brain regions to spreading depolarizations.

RESULTS

Optical imaging experiments in mice showed that both cortical spreading depression and anoxic depolarization preferentially arose in the whisker barrel region of parietal cortex. Intact skull preparations confirmed that this finding was not an artifact of surgical approach, and magnetic resonance imaging recordings demonstrated that the spreading depolarizations did not propagate from a subcortical source. Potassium concentrations showed larger increases in barrel cortex, suggesting a mechanism for the susceptibility. Sensory stimulation biased the timing but not the location of spreading depolarization onset. In humans with traumatic brain injury, there was a trend toward increased incidence of

spreading depolarizations in parietal/temporal sensory cortex compared to other regions.

INTERPRETATION

Spreading depolarizations are preferentially inducible in primary sensory cortex in mice and most likely in humans. This tropism might explain the predominant sensory phenomenology of migraine aura. It also demonstrates that sensory cortices are an at-risk region in brain injury.

FUNDING

International Headache Society Research Fellowship (VBB); National Institutes of Health NS 085413, US Army CDMRP Peer Reviewed Medical Research Program (KCB); US Army CDMRP PH/TBI Research Program (JAH).

Spreading Depression and Migraine Pain as Evolutionary Epiphenomena of TBI

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It has been proposed that spreading depression (SD) and subsequent migraine pain must have been beneficial through evolution to be so prevalent today (Loder 2002). But like neuropathic pain, migraine pain imparts agony and prolonged incapacitation, so the survival advantage is debatable. We propose that migraine pain is a downstream epiphenomena reflecting the higher brain's ability to generate spreading depolarization, a process we suggest can be better rationalized as improving evolutionary fitness. Spreading depolarization evoked by traumatic brain injury (TBI) has likely existed through vertebrate evolution. It causes electrical silence with vasoconstriction in higher gray matter to preserve metabolic energy, to avoid epileptiform activity and to reduce hemorrhage. We suggest that a local increase in metabolic demand that initiates migraine aura (e.g., by a microembolism) is misinterpreted as hemorrhage caused by TBI. The response to TBI (but not to ischemia caused by stroke or heart failure) has had time to evolve as a strategy whereby gray matter depolarizes and vasoconstricts. Both responses characterize SD. If this idea is correct, it follows that lower brain regions more protected from TBI should not generate SD.

Coronal slices from adult rat brain were superfused with artificial CSF. SD was evoked by elevated KCl replacing equimolar NaCl. Single neurons were whole-cell current clamped. Brief exposure (100 seconds) of higher neurons (neocortical, hippocampal, striatal or thalamic) to 52 mM [K⁺]_o resulted in depolarization reaching a brief plateau between -30 and -20 mV. Then a second more rapid depolarization to near zero millivolts indicated

SD generation. Upon return to control aCSF, membrane potential recovered. In contrast, 7 types of neurons recorded in the lower brain (hypothalamus and brainstem) all depolarized to between -30 and -20 mV in elevated K⁺, as expected from the Nernst equation. However, all failed to generate SD. The lack of SD in lower brain gray matter was confirmed by imaging changes in light transmission.

These data indicate that SD is not a default of all CNS neurons. In the higher brain, SD may be the price of adapting to, and dealing with, TBI. An additional but more devastating trade-off to evolving TBI-evoked spreading depolarization is that when our modern brain senses reduced blood flow during stroke or heart failure, it vasoconstricts.

Mechanisms underlying spreading depolarization-induced dendritic beading

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Spreading depolarizations (SDs) are waves of sustained neuronal and glial depolarization that propagate massive disruptions of ion gradients through the brain. SD is associated with migraine aura and recently recognized as a novel mechanism of injury in stroke and brain trauma patients. SD leads to neuronal swelling as assessed in real time with 2-photon laser scanning microscopy (2PLSM). Pyramidal neurons do not express aquaporins and thus display low inherent water permeability. Yet, SD rapidly induces focal swelling (beading) along the dendritic shaft by unidentified molecular mechanisms. To address this issue, we induced SD in hippocampal slices by focal KCl-micro-injection and visualized the ensuing dendritic beading by 2PLSM.

during their translocation mechanism may generate dendritic beading independently of osmotic forces.

We confirmed that dendritic beading failed to arise during large (100 mOsm) hyposmotic challenges, underscoring that neuronal swelling does not occur as a simple osmotic event. SD-induced dendritic beading was not prevented by pharmacological interference with the cytoskeleton, supporting the notion that dendritic beading may entirely result from excessive water influx. Dendritic beading was strictly dependent on the presence of Cl⁻ and accordingly, combined blockade of Cl⁻-coupled transporters, in addition to lactate transporters, led to a significant reduction in dendritic beading without interfering with SD. Furthermore, our in vivo data showed a strong inhibition of dendritic beading upon pharmacological blockage of these cotransporters. We propose that SD-induced dendritic beading takes place as a consequence of the altered driving forces and thus activity for these cotransporters, which by transport of water

Quad Lumen Bolt: Novel technique for minimally invasive monitoring of multiple physiological variables in patients with acute brain injury

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OBJECTIVE

Our previous work validated the detection of cortical spreading depression (CSD) using depth electrodes, when inserted with the well-established strip electrode during craniotomy. We now aim to introduce a novel minimally invasive technique for CSD detection by inserting the depth electrode through a new quad lumen bolt, along with three other probes. These would allow measurements of brain temperature and tissue oxygen; glucose, lactate, potassium; and intracranial pressure (ICP), through, the Licox, Microdialysis and the ICP probes, respectively. This would provide access to a larger patient group for studying CSD, who do not require a craniotomy. Our goal is to implement this technique clinically to allow early treatment of the detected abnormality in critically ill patients with brain injury.

METHOD

Insertion of any three probes, but not all four was possible with the original bolt design. After bench testing insertion of our four probes, we devised a novel technique that required modifications to the original design. We requested the manufacturers to provide us with some additional accessories, and with certain changes to the technique, the redesign enables insertion of all four probes to their required depth.

RESULTS

Our request was met. We suggest the depth electrode to pass through the largest pigtail,

with the pre-marked distance of 16.5cm from the cranial end, to achieve the required depth of 1.8cm into the brain. The ICP probe is to be inserted into the intermediate pigtail, to the pre-marked distance of 16.2cm from the cranial end, to achieve the depth of 3cm. Licox is inserted with an introducer into the second intermediate pigtail that requires attachment of a 1cm extension first, to achieve the depth of 1.5cm. We suggest Microdialysis to insert into the smallest pigtail without an introducer and to rely on the firm white tip for penetration into the brain. An additional 65mm extension needs to attach to this pigtail first to achieve the depth of 1.3cm. All insertions require prior durotomy and pialotomy with a stylet (separate narrower stylet required for the smallest lumen).

CONCLUSION

We will apply our modified technique in the human brain to determine if all variables are detected. We are able to confirm the accuracy of measurements using the 3D Modelling software to ensure that the depths penetrated by each probe are as intended.

SUPPORT

Supported by Wellcome Trust Department of Health (England and Wales) Health Innovations Challenge Fund (Dept. Health Ref: HICF-1010-o80; Wellcome Trust Ref: WT094912).

Multimodal integration and on-line presentation of ICU data using the Moberg ICU monitor: A case of multiple cortical spreading depolarizations in an intracerebral haematoma patient.

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BACKGROUND

Multimodal presentation of data in the evaluation of the neurointensive patient is a challenge, since recordings are made in different platforms. The Moberg ICU monitor provides full integration of multiple datasets and the presentation includes a programmable data interface.

OBJECTIVES

To integrate electrophysiological data with measurements of tissue oxygen tension, ICP, CPP and other clinical data in a patient with spontaneous intracerebral hemorrhage (ICH).

METHODS

A 64 y.o. patient suffering an ICH was operated and had a 6-electrode subdural platinum strip and a LICOX-probe implanted. Effective monitoring time was 109 hours. At the neurointensive care unit vitals from Phillips Intellivue were simultaneously integrated into the Moberg device.

RESULTS

A total of 246 CSDs were recorded. Simultaneous presentation of physiological variable showed affected oxygen tension during CSDs and was clearly demonstrated on the Moberg monitor.

CONCLUSION

The Moberg ICU monitor provides multiple options for integration of ICU data bedside. The system is user friendly to set-up and

makes data handling convenient and reliable - due to time synchronizing. However, the Moberg solution would benefit from a more manageable and dedicated external software, to facilitate the review of the collected data from distant systems (eg. office computers). In this regard, a software plugin for LabChart or a similar dedicated end user program capable of handling and integrating both electrophysiological and non-electrophysiological parameters would make the product complete and support the underlying idea of multimodal monitoring.

Optical imaging of tissue pH changes with spreading depolarization in the intact and ischemic rat brain.

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OBJECTIVES

Spreading depolarizations (SDs) may exacerbate ischemic injury via related atypical hemodynamic responses, but their consequences on tissue pH changes are not yet understood. The regulation of pH of neurons is crucial, because acid loading makes them susceptible for injury. Therefore we set out to compare intracellular pH changes associated with SDs propagating across the intact and the ischemic cortex of rats, visualized by live optical brain imaging.

METHODS

A closed cranial window was mounted over the right parietal bone of isoflurane anesthetized adult male Sprague-Dawley rats ($n=13$). The cranial window incorporated a glass capillary through which $1\ \mu\text{l}$ 1M KCl was applied topically for SD elicitation. Neutral red (NR), a pH sensitive fluorescent dye was injected i.p. (35mM, $2 \times 1\ \text{ml}$) 30 min before the start of image acquisition. Following a baseline period of 50 min, transient global forebrain ischemia was created by 60 min of bilateral common carotid artery occlusion (2VO). SDs were elicited at 15 min intervals prior ischemia induction and during ischemia. Two separate CCD cameras, synchronized to suitable illumination, were used for the dual imaging of changes in cerebral blood flow (CBF, laser speckle contrast imaging) and intracellular pH (NR fluorescence intensity). The pH signal was validated with the use of traditional pH-sensitive electrodes in a separate set of rats.

RESULTS

All SDs were coupled with hyperemic CBF responses, with lower amplitude (48 ± 10 vs. $155 \pm 23\%$) and longer duration (279 ± 55 vs. 163 ± 21 s) during ischemia. SD-associated pH changes were biphasic (initial acidosis followed by transient alkalosis) during baseline, and monophasic (dominant acidosis) during ischemia. The amplitude of acidosis was almost 3 times higher for ischemic as compared with baseline SDs ($\Delta\text{F}/\text{F}$ 0.37 ± 0.04 vs. 0.13 ± 0.02), the ischemic value considerably exceeding the peak of the 2VO induced acidosis ($\Delta\text{F}/\text{F}$ 0.25 ± 0.05). The duration of acidosis tripled for ischemic as compared with baseline SDs (155 ± 33 vs. 45 ± 3.5 s).

CONCLUSIONS

Marked tissue acidosis correlates with the extent of brain injury, and has been traditionally considered as a damaging component of cerebral ischemia. On the other hand, mild acidosis appears to be neuroprotective by delaying SDs and limiting ischemic injury. We propose that the SD-associated, prominent, transient acidosis superimposed on ischemic acidosis worsens tissue survival, thereby rendering SDs malignant in cerebral ischemia. In contrast, the less pronounced pH changes with SDs in the non-ischemic cortex are suggested not to be harmful.

FUNDING

Hungarian Scientific Research Fund (K111923), János Bolyai Research Scholarship of the Hungarian Academy of Sciences, Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-1/13.

Calcium, oxygen and vascular responses to cortical spreading depression and sensory stimulation in mouse model of familial hemiplegic migraine type 1

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Familial hemiplegic migraine type 1 (FHM1) is a rare, autosomal, dominantly-inherited subtype of migraine with a prolonged aura of visual and sensory symptoms. The disease is caused by mutations in the CACNA1A gene, which encodes the α_1 -subunit of CaV2.1 (P/Q-type) voltage-gated Ca^{2+} channels in presynaptic terminals. The migraine aura is most likely caused by cortical spreading depression (CSD), a transient, slowly propagating, depolarization wave, which depolarizes both neurons and glial cells.

In transgenic knockin FHM1 mice that carry a pathogenic R192Q missense mutation, an increase in Ca^{2+} influx in presynaptic terminals increases the probability of glutamate release at pyramidal cell synapses; whereas inhibitory neurotransmission remains unchanged at fast-spiking interneuron synapses in these mice (Tottene et al., 2009). The neuronal circuit that dynamically maintains a tight balance during cortical activity is most likely altered in FHM1 (Vecchia et al., 2014). However, to date, no study has investigated how the FHM1 mutation alters neurovascular coupling calcium activity in vivo.

In the present study, we used a mouse model of FHM1 carrying the R192Q missense mutation to investigate whether the Ca^{2+} activities of neuronal and astroglial cortical cells reflected the effects of the FHM1 mutation on neurotransmission under physiological conditions and during CSD in vivo. Furthermore, we determined whether the altered neurotransmis-

sion was involved in mechanisms that couple synaptic activity to cerebral blood flow (CBF) and to the cerebral metabolic rate oxygen requirement (CMRO₂). The FHM1 mutation led to impaired control of activity-dependent rises in the Ca^{2+} activities of neurons and astrocytes in vivo, as well as an impaired neurovascular coupling. During CSD, FHM1 mice showed a mismatch between O₂ consumption and supply. It resulted in protracted tissue anoxia in FHM1 mice, but WT animals only displayed a reduced level of tissue O₂. Following the spreading depression, FHM1 mice showed a slower recovery of cerebral blood flow to pre-CSD baseline values than wild-type animals. Furthermore, after the CSD, evoked CBF and Ca^{2+} responses were moderately decreased in WT mice, but severely affected in FHM1 mice. Prevention of tissue anoxia and cortical calcium impaired activity in FHM1 may reduce the prolonged impairment of cortical function after migraine attacks.

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A role of the sodium pump in the inverse hemodynamic response to spreading depolarization in rats

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In rats, spreading depolarization (SD) induces vasodilation/hyperemia in naïve tissue but the inverse response when artificial cerebrospinal fluid (ACSF) is topically applied to the brain containing (i) an NO-lowering agent and (ii) elevated K^+ . The inverse response is characterized by severe vasoconstriction/ischemia. The perfusion deficit runs together with the depolarization in the tissue (= spreading ischemia (SI)). Here, we found that pre-treatment with ACSF containing elevated K^+ in vivo led to a selective decline in α_2/α_3 $Na^+/K^+-ATPase$ (NaKA) activity, determined spectrophotometrically ex vivo. Moreover, SI resulted from ACSF containing an NO-lowering agent in combination with the NaKA inhibitor ouabain at a concentration selectively inhibiting α_2/α_3 activity. Decline in α_2/α_3 activity results in increased Ca^{2+} uptake by internal stores of astrocytes, vascular myocytes and pericytes since Ca^{2+} outflux via plasmalemmal Na^+/Ca^{2+} -exchanger declines.

Augmented Ca^{2+} mobilization from internal stores during SD might enhance vasoconstriction and, thus, contribute to SI. Consistently, SI was significantly shortened when intracellular Ca^{2+} stores were emptied by pre-treatment with thapsigargin, an inhibitor of the sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA).

These findings might have relevance for clinical conditions, in which SI occurs such as delayed cerebral ischemia after subarachnoid hemorrhage.

Dynamics of Ionic Shifts in Cortical Spreading Depression

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The mechanisms underlying the propagation of cortical spreading depression (CSD) are poorly characterized. The slow speed of the CSD wave implies that it is mediated by diffusion of a chemical substance, yet the identity of this substance and the pathway it follows are unknown. Intercellular spread between gap junction-coupled neurons or glial cells and interstitial diffusion of K⁺ or glutamate have been proposed. I will present and dis-

cuss recent electrophysiological and 2-photon imaging data on the spatiotemporal dynamics of ionic shifts at the CSD wavefront, including data obtained in fully awake mice.

The data argue against intercellular spread of Ca²⁺ carrying the CSD wavefront and are in favor of interstitial K⁺ diffusion, rather than glutamate diffusion, as the leading event in CSD.

Preliminary results on detection of spreading depolarisations using near infrared spectroscopy

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ABSTRACT

Spreading depolarisations (SD) occur in about 50-60% of the patients suffering from severe traumatic brain injury(1). During this phenomenon there is an interruption of normal cortical function due to a severe alteration of the ionic fluxes (intracellular calcium increases, extracellular potassium increases). The

healthy neurons of the injured brain respond to this ionic redistribution by dramatically increasing their metabolic demands(2), hence local blood flow increases. Failure to reverse the ionic imbalance leads to terminal depolarisation and hence an extension of the injury. The neurovascular coupling(3) following activation is usually (...)

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associated with a localised transient increase of the Oxy-Haemoglobin (Oxy-Hb) and a reduction of the Deoxy-Haemoglobin (Deoxy-Hb), these changes can be detected non-invasively by near infrared spectroscopy (NIRS) (4). Hence NIRS might detect SDs.

METHODS

We are recruiting patients at St Mary's Hospital, the inclusion criteria are:

- Aged > 18, < 80
- Head Injury resulting in period of intensive care monitoring > 24 hours

The exclusion criteria are:

- Bilateral extra-axial blood collections.
- Extensive scalp injury that would inhibit the placement of NIRS cap
- Devastating head injury not expected to survive > 24 hours

The system used is the HITACHI ETG 4000, which applies two wavelengths 695nm and 830nm creating up to 52 channels, the inter-optode distance is 3cm and determined by head-mounted holders. Treatment of the severely brain injured patients follows standard hospital procedures. The position of the optodes relative to the patient's skull is based on the results of the CT brain and on the 10-20 system (commonly used in EEG). We used 24 channels except from two sessions where we used 48 channels. The intracranial pressure was monitored in line with standard practice. The patients were sedated and intubated and variations of their heart rate, position, ventilation and blood pressure were noted. Increases of intracranial pressure more than 20mmHg were also noted on the Hitachi system. According to the protocol the duration of each measurement epoch is 60 minutes followed by 15 minutes of resting period in order to avoid possible skin injury.

RESULTS

We have monitored 7 patients with a total recording time of 50 hours. There was no evidence of skin damage caused by the NIRS probes. In three TBI patients who had not had craniotomy we saw transient increases in Oxy-Hb coupled to a transient decrease in deoxyHb. The duration of these events was variable, but typically between 3-8 mins. On several occasions these events repeated. The inter event time interval was variable but typically 18-25

mins. One of these patients subsequently had craniotomy, events were still seen but with increased noise due to artefact. We tentatively suggest that these results are consistent with the non-invasive detection of SD in the injured human brain. Preliminary results indicate spontaneous increase of the blood flow over the injured side of the brain which may represent an indication of active neurovascular coupling, as there were no other physiologic stimuli which could explain the spontaneous increase of the blood flow over the injured brain. Further investigation is under way in order to validate these promising results by simultaneously detecting the SDs invasively using ECoG.

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Oxygen tensions measured at baseline and before spreading depolarization do not evidence a supply-demand mismatch in patients with aneurysmal subarachnoid hemorrhage

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It has recently been reported that compared with baseline tissue partial pressure of oxygen (ptiO₂) in the absence of spreading depolarization (SD), average ptiO₂ immediately preceding SD was significantly lower in 7 patients with malignant hemispheric stroke (MHS)¹. In contrast, there was no difference between baseline and pre-SD segments in terms of mean arterial pressure (MAP) or intracranial pressure (ICP), suggesting that O₂ supply-demand mismatch predisposes to SD in patients with MHS. Here, we investigated whether the same relationships exist in patients with aneurysmal subarachnoid hemorrhage (aSAH). We performed a prospective study in 33 patients, using ptiO₂ measurements simultaneously with near direct current/alternating current-electrocorticography (ECoG).

During a total recording time of 7397.6 hours, 31 of 33 patients had a total of 1305 SDs. In 1140 SDs, simultaneous ECoG and ptiO₂ data were available. We did not find a significant difference between baseline and pre-SD segments regarding ptiO₂ (31.5 ± 10.2 vs. 29.3 ± 12.2 mmHg, $P=0.118$, Wilcoxon Signed Rank Test), but a significant difference in terms of cerebral perfusion pressure (82.7 ± 8.9 vs. 74.6 ± 14.3 mmHg, $P=0.06$, Wilcoxon Signed Rank Test) and MAP (96.4 ± 9.1 vs. 89.2 ± 12.9 mmHg, $P<0.001$, Wilcoxon Signed Rank Test). ICP and brain temperature did not differ significantly. We observed 874 SDs in 26 patients with an initial ptiO₂ decrease, 13 SDs in 5 patients with an initial increase and 253 SDs in 27 patients with no change of ptiO₂. The

average ptiO₂ preceding SD was not significantly different between the 3 types of ptiO₂ responses in the 5 patients who displayed all 3 response types (23.3 ± 9.8 vs. 25.4 ± 17.5 vs. 23.7 ± 10.5 mmHg, Friedman Repeated Measures Analysis of Variance on Ranks). In conclusion, we did not find any evidence for an O₂ supply-demand mismatch predisposing to SD occurrence in patients with aSAH. Interestingly, no evidence was found that the baseline level of ptiO₂ determines the ptiO₂ response type to SD.

ivon Bornstädt et al (2015) Neuron 85:1117-31

Cortical spreading depolarizations in patients with intracerebral hemorrhage – preliminary data

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INTRODUCTION

Perihematomal edema expansion (PHE) contributes to increased morbidity and mortality after spontaneous intracerebral hemorrhage (ICH). Pathophysiology of PHE is incompletely understood. Recently, the role of cortical spreading depolarizations (CSDs) in secondary brain injury was established in subarachnoid (SAH) and traumatic brain injury (TBI) patients. However, the value of CSDs after ICH is not known.

METHODS

Patients with ICH fulfilling the inclusion criteria were prospectively enrolled in the observational COSBID study (Co-operative Study on Brain Injury Depolarisations). g.BSamp (g.tec, Austria) connected to PowerLab and LabChart software (Adinstruments) was used for electrocorticography (EcoG). Electrocardiogram patches at the patients shoulder and bed served as groundings, a surface reference electrode was glued on the mastoid. The duration of EcoG-depressions was defined as the time between depression onset and start of EcoG recovery in the integral of power calculations (0.5-45Hz; 60 sec time constant decay). Brain tissue oxygen tension (PbtO₂), cerebral blood flow (CBF), cerebral metabolism and intracranial pressure (ICP) were monitored in the PHE region. Data are presented as median and interquartile range.

RESULTS

21 patients were analyzed. Hematoma evacuation (ICH volume: 54 [33-69] ml) was performed in 20 patients, 1 subject underwent craniectomy. Patients were 61 (56-73) years old and 43% female. Monitoring time per patient was 8 (4-12)

days. A total of 184 CSDs with 17 (10-31) minutes EcoG depression were observed. 84% (N=15) of patients showed marked expansion of PHE by 25 (10-50) ml within 3-6 days after bleeding. CSDs occurred in 73% (N=11) of patients with PHE expansion. Interval between CSDs was 90 minutes (33-230). CSDs were associated with a significant decrease of PbtO₂ (-4mmHg [-2;-6]; duration 12[5-23] minutes) in 59% (74/126). CBF changes in 98% (47/48) and metabolic derangement in 80% (80/100) of CSDs. PHE expansion was observed in all patients with spreading convulsions (N=4) and patients with repetitive CSDs occurring as clusters (N=5). DISCUSSION: CSDs are common in ICH patients and are associated with perihematomal PbtO₂ and CBF decreases and metabolic derangement. Clusters of CSDs might be associated with detrimental changes of the perihematomal brain tissue.

Cerebral Autoregulation and Cortical Spreading Depolarizations in Patients with Spontaneous Intracerebral Hemorrhage

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BACKGROUND

Multimodal neuromonitoring including monitoring of cortical spreading depolarizations (CSDs) provides insights into patho-physiologic processes leading to secondary brain injury following intracerebral hemorrhage (ICH).

OBJECTIVES

We intended to investigate known indices for cerebral autoregulation in relation to the occurrence of CSDs.

METHODS

Data from 18 prospectively recorded ICH-patients with invasive neuromonitoring including intracranial pressure (ICP), brain tissue oxygen tension (PbtO₂), cerebral blood flow (CBF) and electrocorticography (EcoG) were analyzed. Cerebral autoregulation indices were calculated using 300sec moving Pearson's correlation coefficients of ten seconds trend values between cerebral perfusion pressure (CPP) and PbtO₂ (oxygen reactivity index, ORx), mean arterial pressure (MAP) and ICP (pressure reactivity index, PRx) and MAP and CBF (CBFrx). Impaired cerebral autoregulation was defined as an index of >0.2. Statistical analysis was performed using generalized estimation equations. Data are presented as median and interquartile-range (IQR).

RESULTS

Median ICH volume was 48cc (IQR, 32-70), median age was 61 years (IQR, 56-73). Cerebral autoregulation monitoring was available over 40 hours (IQR 14-54) per patient which included 163 CSDs (median of 7 CSDs/patient (3-15)).

Impaired cerebral autoregulation was detected in all ICH-patients during 24%-73% (range) of the available autoregulation data. Cerebral autoregulation was impaired in 45% (N=73) preceding the CSDs and remained impaired in 99 % (N=72/73) after CSDs. In CSDs with baseline intact autoregulation (55%, n=90/163), autoregulation indices changed to impaired status following CSDs in 21% (N=19; P<0.0001). Impaired cerebral autoregulation preceding CSDs was associated with prolonged depression of cortical activity [25min (IQR, 14-35) versus 13min (IQR, 9-24); P<0.001] and more severe CBF-drops during CSDs [-10ml/100g/min (-13 to -6) versus -3ml/100g/min (-18 to 10); P=0.02]. PbtO₂ significantly decreased following CSDs onset independent of baseline autoregulation capacity.

CONCLUSION

Impaired cerebral autoregulation was associated with a significantly prolonged depression of cortical activity and more profound hemodynamic response to CSDs in patients with ICH.

On-line microdialysis monitoring of COSBID patients – detecting the neurochemical consequences of spreading depolarisation.

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ABSTRACT

The depolarisation phase of a spreading depolarisation (SD) represents perhaps the most intense challenge possible to flow metabolism coupling in the injured brain. We have shown previously using a microdialysis probe placed in human peri-ilesional cortex and rapid sampling microdialysis that extracellular glucose is driven down in response to SD (1). As part of a new Wellcome Trust funded clinical instrument we now present the first clinical data from a new microfluidic analysis system giving real-time measurement of dialysate potassium, glucose, lactate and pyruvate.

METHODS

Patients were recruited to King's College Hospital according to COSBID selection criteria. A microdialysis probe (M Dialysis CMA70) was placed obliquely in the injured cortex and connected to our new bedside microfluidic analysis unit via a low volume connection tubing. Dialysate analysis was by sensors and biosensors placed in a ultra-low volume microfluidic chip. Analytes included: *Potassium* – micro ion selective electrodes (320 micron od) Glucose and lactate – were detected using 360 micron needle electrochemical cells configured as amperometric biosensors using glucose and lactate oxidase respectively. Pyruvate – a microfluidic solution was devised using dosing of a custom enzyme / cofactor cocktail followed by amperometric detection. The instrument was completed by a computer controlled auto-calibration that operated each 3h while the system was in the intensive care unit.

RESULTS

The system has been in use in the intensive

care unit at King's College Hospital since Jan 2015. Once set up the instrument run unattended although occasionally micro-bubbles cause transient noise spikes. Spontaneous SD's in the human brain result in a clearly resolved neurochemical signature sharing many features of that seen in a proof of concept study in the rat (2). The sharp increase in dialysate potassium level indicates the start of an SD in the dialysis stream, lasting for 2-5 mins. Very clear glucose decreases then follow on a similar timescale. Lactate levels show strong transient increases, with the precise time courses varying between patients. The lactate / glucose ratio shows a strong transient increase from typically 0.2 to >5 for period of 5 mins. A full analysis of the latest clinical data will be given. Support: Supported by Wellcome Trust / Department of Health (England and Wales) Healthcare Innovation Challenge Fund Grant number HICF-0510-080

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Automating detection and analysis of depolarisations and other secondary insults to the injured human brain

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ABSTRACT

Some three and one half years ago we set out to build a new class of clinical instrument specifically designed to automatically detect the occurrence of spreading depolarisations and other dynamic secondary insults, and to quantify the effect of such insults on the injured human brain. This abstract gives a progress report in light of data from proof of concept patient recordings made in King's College Hospital.

METHODS

Patients were recruited to King's College Hospital according to COSBID selection criteria. We describe each part of the system in turn: Electrocorticography – the system electronics records from depth electrode arrays (i) (Adtech) placed into cortex and/ or COSBID classical strip electrodes placed during craniotomy. A low-noise battery powered head-stage carries out pillow side digitisation at 24bit resolution before transmission to a bedside unit. Neurochemical changes – A microdialysis probe placed obliquely in the injured cortex is connected to a new bedside on-line microfluidic analysis unit (described elsewhere at this meeting) to give continuous recording of brain extracellular potassium, glucose, lactate and pyruvate levels. Parenchymal oxygen is monitored continuously in white matter using a LICoX probe. Physiological parameters – our real-time data set is completed by patient pulsatile ABP and ICP, collected using iXtrend software.

RESULTS

Real time event detection– In collaboration with our partners Cybula we have ported our

off-line secondary insult detectors to a new real-time system running at the bedside. SD's are detected from both depth (unipolar) and strip electrodes (bipolar) and parameterised to allow future analysis. Detectors also detect transient hypotension and transient dips in brain PbtO₂ Work is in progress to detect transient falls in brain glucose and increases in brain potassium. All detectors run with excellent sensitivity and specificity (as judged by ROC curve analysis).

Event visualisation – A sub-project lead by Dr Clemens Pahl has worked on optimisation of the visual interface for the intensive care unit. For each detector channel false colour bins indicate the occurrence and severity of an adverse event or level.

SUPPORT

Supported by Wellcome Trust / Department of Health (England and Wales) Healthcare Innovation Challenge Fund Grant number HICF-0510-080

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The proinflammatory cytokines TNF α and IL-1 β diminish amplitudes of cortical spreading depolarization (CSD) in adult rats – is there a common principle of neuroprotection?

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It is known that the cytokines TNF α and IL-1 β are released in the brain in pathophysiological states such as stroke and neurodegenerative diseases. We tested, whether these cytokines could participate in neuroprotection via influencing CSD. CSD were recorded in spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) in cerebral cortex with two pairs of glass micropipettes (distance 5–6 mm) at depths of 1200 μ m. CSD was elicited by a microinjection of 1 M KCl (tip diameter 5 μ m, 100 kPa, 300 ms – 1 s). One area of the cortex was separated by a wall and treated with the cytokine, in the other untreated one CSD was elicited. In the TNF α group we applied either different doses of TNF α or TNF α together with an antagonist to the TNF α receptor 1 or 2, or TNF α together with the GABA $_A$ receptor antagonist bicuculline. In the IL-1 β group, we applied either different doses of IL-1 β alone to the cortical surface, or IL-1 β together with an IL-1 receptor antagonist. We also applied IL-1 β together with bicuculline. In a subgroup, the cortices treated with IL-1 β were stained with Evans blue, and we looked for plasma extravasation and for the localization of the IL-1 β receptor. TNF α dose-dependently decreased the CSD amplitudes, and 5 ng TNF α slowly abolished CSD waves in the treated area. These effects were dependent on the TNF receptor 2, but not on the TNF receptor 1 because they were blocked specifically. The target of TNF α were inhibitory interneurons which expressed the TNF receptor 2. The blockade of the GABA $_A$ receptor with bicuculline prevented the reduction of the CSD amplitudes by TNF α . IL-1 β decreased the CSD amplitudes in the treated area, but did not abolish CSD waves completely. The strongest reduction of CSD was observed with 10 ng of

IL-1 β , but a typical dose-dependency of the amplitude effects did not exist. Coapplication of IL-1 β with the IL-1 receptor antagonist prevented the reduction of CSD amplitudes; they rather increased after treatment. The GABA $_A$ receptor antagonist bicuculline alone slightly increased CSD amplitudes. Coapplication of bicuculline together with IL-1 β enhanced CSD amplitudes as well, suggesting that inhibitory neurons may also be activated by IL-1 β . Histological sections showed the expression of IL-1 β receptors at neurons and microglia and plasma extravasation in the IL-1 β treated areas. These results show that both TNF α and IL-1 β decrease CSD, presumably via the release of GABA. This action might contribute to neuroprotection. The plasma extravasation induced by IL-1 β , however, might be harmful for the brain that is already damaged by e.g. stroke. Therefore IL-1 β cannot be classified as a neuroprotective cytokine in the brain.

Effects of moderate hypothermia on spreading depolarization in the gyrencephalic brain.

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INTRODUCTION

The effects of hypothermia on spreading depolarization (SD) are controversially discussed in the literature. We evaluated the electrophysiological characteristics as well as the hemodynamic response of SD during moderate hypothermia and compared to normothermia in an experimental pig model.

MATERIAL AND METHODS

After intubation and sedation of 10 pigs, large craniotomy with dura opening was performed to induce SD using 1mol/l KCl solution on a preconditioned cortex. Electrocorticography and intrinsic optical imaging detected events of SD. Hypothermia was induced using body surface cooling and SD induced before hypothermia during normothermia (36.0–37.0°C, rectal temperature), and moderate hypothermia (33.0–33.5°C), and after rewarming during normothermia (36.0–37.0°C). The amplitude and duration of SD events as well as the hemodynamic response was compared between normothermia and hypothermia.

RESULTS

SDs in ECoG during moderate hypothermia showed to decrease significantly in amplitude compared to normothermia ($77\% \pm 5.2$ vs $96\% \pm 3.8$; $p=0.036$) and in velocity (4.5 ± 2.5 minutes vs 2.5 ± 1.3 minutes; $p<0.001$). In iOS, SDs during moderate hypothermia were observed in 100% of the cases. Nevertheless SDs had a decrease in amplitude ($p=0.004$), hyperemia ($p=0.011$), oligemia ($p=0.004$) and total duration ($p<0.001$).

CONCLUSIONS

Moderate hypothermia during SD shows to be effective in decreasing amplitude and velocity measured by ECoG. In iOS SDs blood volume changes and duration were significantly decreased, suggesting that moderate hypothermia might serve as a form of protection during SD.

Cellular mechanisms of supply-demand mismatch during spreading depolarization.

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Following stroke or traumatic brain injury, repetitive waves of spreading depolarization (SD) can contribute to progressive expansion of neuronal injury. Excessive glutamate release and severe postsynaptic Ca^{2+} accumulation occur during SD, and failure of neuronal recovery after SD is likely determined by adequacy of metabolic substrates required to reestablish ionic gradients. Objective: To use animal models to investigate cellular mechanisms underlying neuronal energy demand and resupply following SD, as a step towards development of interventions to reduce damaging consequences of SD in injured brain. Methods: In vitro studies were in murine brain slices and in vivo recordings were in neocortex of anesthetized mice. Glutamate and Ca^{2+} were assessed with fluorescent indicators, including genetically-encoded sensors and visualization of co-stored synaptic Zn^{2+} as a surrogate for glutamate.

Vascular dynamics were assessed either in brain slices assessing arterioles pre-constricted pharmacologically to a physiologic level of tone, or in vivo, using laser speckle contrast imaging (LSCI). SDs were generated by focal KCl microinjection, or as a result of distal middle cerebral artery occlusion. Results: Zn^{2+} and glutamate imaging demonstrated waves of excessive transmitter release time-locked to SD, and complement recent glutamate microelectrode evidence that transmitter release in peri-infarct regions is restricted to SD events. Neuronal Ca^{2+} transients during SD were prolonged by metabolic depletion of individual neurons in vitro, and neurons located more distant from supplying arterioles demonstrated longer-lasting Ca^{2+} transients during SD in vivo. In vitro, vasoconstrictor

responses to SD could be blocked by pre-incubation with nicardipine, an L-type voltage-dependent Ca^{2+} channel antagonist. Preliminary experiments showed that the vasoconstrictive component of blood flow transients during peri-infarct SDs was completely abolished by focal application of nicardipine. Conclusions: Together with microelectrode glutamate recordings, these data emphasize the episodic nature of glutamate release to excitotoxic signaling in injured brain. NMDA receptor antagonism may be more effective if limited to patients with demonstrated SDs, and with drug delivery limited to times when SDs are detected. Such an approach may limit deleterious effects of long-term receptor antagonism on recovery and repair mechanisms. In addition, L-type blockers may be useful for improving vascular resupply after SD, particularly if significant central drug concentrations can be achieved without significant peripheral effects.

Acknowledgements: Supported by NIH grant NS51288

Effect of a subanesthetic dose of ketamine on spreading depolarization after experimental subarachnoid hemorrhage in mice

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Spreading depolarization (SD) is an intensive neural and glial depolarization wave that propagates across the cerebral cortex. It has been implicated in the pathophysiology of ischemia induced by subarachnoid hemorrhage (SAH) and other brain injuries. Studies have demonstrated that ketamine has the capacity to modify SD occurrence in normal conditions. However, few studies have demonstrated the effect of ketamine in SAH. In the current experiment, the effect of subanesthetic dose of ketamine (30mg/kg) on SD recorded with intrinsic optical signal imaging (IOS) after experimental SAH was studied in mice. For induction of SAH, one 5-0 nylon monofilament was introduced into the extra carotid artery and advanced into the bifurcation of the middle cerebral artery and anterior cerebral artery, and then this bifurcation was perforated. Subsequently, the position of animals was changed from supine to prone position. A CCD camera with an optical band pass filter (564 nm), connected with a computer, was mounted above the thinned mouse skull to record SD. Meanwhile, ketamine or saline (30 mg/kg) was given every half hour after SAH or sham surgery. For assessment of brain edema, mice were killed 3 hours after SAH or sham by decapitation and brain water content was measured by the wet/dry weight method. It was found that 199 SDs occurred after SAH and 30 SDs occurred in sham groups. Moreover, SDs exhibited 4 distinct types: type I (transient increase), type II (transient increase with subsequent plateau), type III (transient increase with subsequent decrease), and type IV (step increase) in the profile of reflectance intensity change, which may imply different blood volume changes in the cerebral cortex. Com-

pared with the control, the brain water content was significantly higher in the SAH groups. Moreover, the brain water content in ketamine-treated animals was lower than in saline-treated groups, but there was no significant difference. Compared with saline, ketamine was capable of reducing the number of ROIs reached by SD and the intensity change of SD, diminishing the cortex area covered by SD, slowing down SD propagating speed, and decreasing the duration of SD intensity change. However, the occurrence of SD was not prevented by ketamine, which means ketamine at this dosage cannot inhibit SD induction after SAH in mice. This might be caused by the low dose or mode of administration. Another explanation might be that the deleterious condition is stronger than the NMDA receptor antagonist, and thus despite the presence of ketamine, SD still occurs. Our experiments suggest that SD occurs in a high incidence acutely after SAH. As a NMDA receptor antagonist, ketamine may have therapeutic benefit in inhibiting SDs. However, for human use, an appropriate dosage and route of administration should be studied and established.

Clinical interventions targeting CSD: preliminary observations and pilot clinical trials.

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The deleterious effects of CSD after brain injury have been well described and are consistent with the vast majority of animal data. The role of therapeutic intervention remains unclear. The best available data to date is from the retrospective analysis of the COSBID monitoring data, which showed a significant effect of ketamine when compared to other standard sedation regimens in decreasing the frequency of CSD. A number of potential confounders exist in any retrospective study of this nature, however promising reports of frequency of CSD occurrence have noted this effect consistently. Another medication suspected to have a role in either the occurrence of CSD or the deleterious vascular effects is nimodipine, which has been proven to be effective at decreasing morbidity after aneurysmal subarachnoid hemorrhage despite the lack of a significant effect on large vessel vasospasm. We now have clinical protocols at the University of New Mexico to evaluate these two potential targets. We have recently gained approval for a prospective internally controlled trial of ketamine versus other sedation regimen in patients with severe aneurysmal SAH or TBI requiring craniotomy and electrode placement. Patients will receive alternating 6 hour periods of ketamine versus other sedation regimen (usually propofol), both used for standard clinical sedation.

The occurrence of CSD during each period will be tested. In a second trial, patients enrolled in a phase I/II clinical trial and upcoming phase III trial of single dose intraventricular nimodipine versus standard oral nimodipine in patients with aneurysmal SAH (NEWTON) will be enrolled into an observational study of occurrence of CSD. The occurrence of CSD will be measured in patients undergoing craniotomy and strip electrode placement. The stand-

ard oral nimodipine group will be compared to the intraventricular group. Preliminary data from the Phase I/II trial shows markedly higher intraventricular concentration of nimodipine in the intrathecal group compared to controls and evidence of suppression of delayed CSD in 2/3 patients. These pilot data will test the feasibility of clinical trials targeting CSD in select high-risk populations.

How to plan and manage an international multicentre trial?

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The answer is PICO, GCP, avoiding bias, working with a good trial centre – and a lot of hard work... PICO stands for Population, Intervention, Comparator and Outcome; these are the cornerstones of the trial design.

Population means defining the population to whom the intervention should be applied in clinical practice. The P must be well-defined so that conclusions can be drawn, however, if it is too restricted it will not mirror clinical reality and further recruitment of patients will be hampered. The sample size must represent a realistic and clinically relevant effect size. The intervention must be applied in such way, dose and timeframe that the effect is likely to occur, if this collides with patient safety issues – or practical problems – you must ask yourself if the intervention is really worth testing.

The comparator must represent current best practice or be a real placebo/sham intervention to provide solid data. Blinding between I and C – or truly blinded endpoint assessment if this is not feasible – is a must; otherwise you test the investigators trust in the intervention..

Outcome or endpoint is what you want to avoid by applying the intervention, e.g. death. It must be well-defined and relevant to the patient to affect clinical practice.

Protocol and analysis plan must be published before trial initiation. GCP – Good Clinical Practice – is an international code on how to conduct trials with special focus on ethics and data quality. Investigators are required to learn this code – e.g by attending a course – and monitoring aiming at verifying data by is compulsory for data quality and avoiding

of fraud. The trial centre handles case record forms, randomisation, adverse event reporting, statistical analysis – based on the plan from the investigators – and in some cases also monitoring. In Denmark this is best handled in academic trials by the GCP-units of the universities. Training is essential to ensure quality and adherence to protocol in all sites.

And don't introduce too many time consuming questionnaires, patient diaries, frequent blood samples with complicated processing or devices that only work in the hands of the inventor.



COSBID

*Cooperative Studies On Brain
Injury Depolarisation*