Aus dem Institut für Neurophysiologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Blood-brain barrier dysfunction and pharmacoresistance of seizures

zur Erlangung des akademischen Grades
Doctor of Philosophy (PhD) in Medical Neurosciences

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Abstract

Sufficient seizure control can not be achieved in fifty to seventy percent of focal epilepsies, particularly in temporal lobe epilepsy, with present antiepileptic medications. Pharmacoresistance might result from changes in the properties of drug targets, augmented expression of drug efflux transporters, increased severity of seizures, network reorganization and / or development of drug tolerance. Recent studies indicated that blood-brain barrier (BBB) disruption is common among patients with pharmacoresistant focal epilepsies. Following BBB dysfunction, serum albumin can leak into the brain parenchyma, be taken up by astrocytes and / or neurons and trigger various transcriptional changes (e.g. reduction in inward rectifying potassium channel 4.1 (K_{ir} 4.1), water channel 4 (AQP4) and gap junctions) via astrocytic TGFßR-II pathway, and lead to hyperexcitability. Serum albumin transports many hormones and drugs including some of the conventional antiepileptic drugs (AEDs), namely phenytoin (PHT), valproic acid (VPA), carbamazepine (CBZ), and phenobarbital (PHB) with variable binding affinities. We therefore hypothesized that following BBB disruption, albumin interferes with the action of these particular AEDs by binding to the drugs or by inducing transcriptional alterations of their targets.

To test this hypothesis, we first induced seizure-like events (SLEs) by 4-aminopyridine, in acute entorhinal cortex-hippocampal slices from adult rats and investigated the efficacy of conventional AEDs in the presence of albumin by extracellular field recordings. Unbound AED concentrations were detected by ultrafiltration and high-performance liquid chromatography. Next, we applied albumin intracerebroventricularly (icv) 24 hrs prior to the experiments and analyzed the effects of CBZ on acute slices from these albumin-pretreated rats.

Conventional AEDs failed to suppress SLEs in the entorhinal cortex in the presence of albumin. This effect was partially caused by buffering of PHT and CBZ by albumin. A two-fold increase of the maximal therapeutic concentration of CBZ resulted in SLE blockage. In slices obtained from animals pretreated with icv albumin, CBZ suppressed SLEs, similar to its effect in control slices. We also found that in the absence of albumin, application of serum-like electrolytes with increased potassium, decreased calcium and magnesium concentrations, transformed SLEs into late recurrent discharges that were resistant to CBZ.

We therefore concluded that a dysfunctional BBB with acute extravasation of serum albumin into brain's interstitial space and / or alterations in the extracellular electrolyte concentrations could cause pharmacoresistance. Thus, BBB monitoring can guide the treatment to control seizures in patients with BBB dysfunction using AEDs with low albumin binding affinity.

Abstract (German)

Mit den derzeit verfügbaren Antiepileptika (AE), wird keine ausreichende EpilepsieAnfallskontrolle in fünfzig bis siebzig Prozent der fokal Epilepsien, insbesondere der Temporalappenepilepsie, nicht erreicht. Pharmakoresistenz ist möglicherweise Folge von veränderten Eigenschaften der Wirkstoffziele, einer erhöhten Expression von EffluxTransportern, massiver werdenden Anfällen, Reorganisation des Netzwerks oder / und der Entwicklung von Medikamententoleranz. Neueste Studien zeigen, dass eine Blut-HirnSchrankenstörung (BHS) häufig bei Patienten mit pharmakoresistenten fokal Epilepsien vorkommt. Nach BHS kann Albumin in das Hirnparenchym austreten und wird dort durch Astrozyten und ggfs. auch Neuronen aufgenommen. Der astrozytäre TGFßR-II-Signalweg wird aktiviert, woraus Transkriptionsänderungen (z.B. eine verminderte Expression einwärts gleichrichtender Kalliumkanäle 4.1 (K_{ir} 4.1), Wasserkanäle 4 (AQP4) und Gap Junctions) mit nachfolgender Übererregbarkeit resultieren. Viel Hormone und Medikamente binden an Serumalbumin und werden so transportiert. Dazu gehören einige der konventionellen AE – Phenytoin (PHT), Valproinsäure (VPA), Carbamazepin (CBZ) und Phenobarbital (PHB). Wir haben daher die Hypothese aufgestellt, dass nach BHS, Albumin durch direkte Bindung an AE oder durch Induktion von Transkriptionsänderungen des AE-Targets, die Wirkweise der AE stören.

Um diese Hypothese zu testen, wurden zuerst krampfähnliche Ereignisse durch 4-Aminopyridin in Akutschnitten des entorhinalen Kortex und Hippokampus der ausgewachsenen Ratte induziert. In diesen Schnitten haben wir die Wirksamkeit von konventionellen AE in der Gegenwart von Albumin durch extrazelluläre Feldpotenialableitungen untersucht. Die Konzentration ungebundener AE wurden durch Ultrafiltration und Hochleistungsflüssigkeitschromatographie detektiert. In weiteren Experimenten haben wir 24 Stunden vor dem ex-vivo Experiment Albumin intrazerebroventrikulär (izv) appliziert und die Wirkungen von CBZ auf die Akutschnitte aus diesen Albumin-vorbehandelten Ratten analysiert.

Konventionelle AE konnten krampfähnliche Ereignisse im entorhinalen Kortex in Gegenwart von Albumin nicht unterdrücken. Dieser Effekt wurde teilweise durch die Pufferung von PHT und CBZ durch Albumin verursacht. In doppelter maximaler therapeutischer Konzentration, unterdrückte CBZ krampfähnliche Ereignisse. In Schnitten von Tieren, die mit izv Albumin vorbehandelt wurden, unterdrückt CBZ die krampfähnlichen Ereignisse ähnlich wie in den naiven

Schnitten. Die Anwendung von serumartigen Elektrolytkonzentrationen mit erhöhter Kalium-, und verringerter Calcium- und Magnesiumkonzentration in der Abwesenheit von Albumin hat epileptische Entladungen in späte rezidivierende Entladungen umgewandelt, die CBZ-resistent waren.

Wir kommen daher zu dem Schluss, dass eine BHS mit akuter Extravasation von Serumalbumin ins Hirnparenchym oder / und Veränderungen in den extrazellulären Elektrolytkonzentrationen der Pharmakoresistenz zugrunde liegen können. In Fällen von mehrfachem Versagen von AE, könnte die Überwachung der Permeabilität der Blut-Hirn-Schranke helfen erfolgreichere Behandlung zu leiten um mit einem AE mit niedriger Albuminbindungsaffinität Anfälle besser zu kontrollieren.

Affidavit

I, Seda Salar certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Blood-brain barrier dysfunction and pharmacoresistance of seizures”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date _____ Signature

Detailed Declaration of Contribution

Seda Salar had the following share in the following publication:

“Blood-brain barrier dysfunction can contribute to pharmacoresistance of seizures”
Seda Salar, Anna Maslarova, Kristina Lippmann, Julia Nichtweiss, Itai Weissberg, Liron Sheintuch, Wolfram S. Kunz, Zamir Shorer, Alon Friedman, Uwe Heinemann
***Epilepsia*, 2014**

Seda Salar did the operations and the experiments (including revision experiments, except the determination of the free drug concentrations by ultrafiltration and high-performance liquid chromatography), analyzed and interpreted the data (including the data from revision experiments, except the data from free drug concentration analysis), prepared the figures and contributed to the writing of the manuscript. (contribution 70 %).

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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☐	21	CEPHALALGIA	0333-1024	7097	4.891	3.736	0.926	121	7.6	0.01116	0.985
☐	22	MULT SCLER J	1352-4585	7810	4.822	4.451	1.042	236	4.9	0.02142	1.344
☐	23	SLEEP	0161-8105	16335	4.591	5.753	1.026	196	8.5	0.02762	1.986
☐	24	EPILEPSIA	0013-9580	22358	4.571	4.424	0.982	279	7.6	0.04223	1.329
☐	25	BRAIN STIMUL	1935-861X	1920	4.399	4.737	0.949	117	2.9	0.00683	1.431
☐	26	EUR NEUROPSYCHOPHARM	0924-977X	5171	4.369	4.754	0.799	189	5.2	0.01261	1.373
☐	27	EUR J NEUROL	1351-5101	7843	4.055	3.786	0.734	214	5.3	0.02095	1.176
☐	28	J PAIN	1526-5900	6283	4.010	4.577	1.061	115	5.6	0.01901	1.629
☐	29	INT J NEUROPSYCHOPH	1461-1457	4959	4.009	4.533	0.977	175	4.6	0.01369	1.270
☐	30	ALZHEIMERS RES THER	1758-9193	676	3.979	4.364	0.765	68	2.5	0.00330	1.449
☐	31	NEUROREHAB NEURAL RE	1545-9683	3533	3.976	4.626	0.787	89	5.0	0.01091	1.402
☐	32	PARKINSONISM RELAT D	1353-8020	4893	3.972	3.885	0.955	242	4.3	0.01494	1.133
☐	33	NEUROPATH APPL NEURO	0305-1846	2792	3.927	4.183	1.393	61	7.8	0.00521	1.305

☐	34	CURR ALZHEIMER RES	1567-2050	2813	3.889	3.933	0.558	104	4.3	0.00779	1.055
☐	35	BRAIN PATHOL	1015-6305	3976	3.840	3.710	1.029	70	7.2	0.00814	1.236
☐	36	J NEUROPATH EXP NEUR	0022-3069	7949	3.797	4.152	0.549	91	>10.0	0.01091	1.385
☐	37	CEREBROVASC DIS	1015-9770	5770	3.754	3.451	0.371	105	6.3	0.01489	1.201
☐	38	J NEUROSURG	0022-3085	29516	3.737	3.573	0.634	374	>10.0	0.03310	1.125
☐	39	J NEUROTRAUM	0897-7151	10198	3.714	4.056	0.867	196	6.9	0.02055	1.170
☐	40	PROG NEURO-PSYCHOPH	0278-5846	8909	3.689	3.797	1.344	186	5.9	0.01805	1.001

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Epilepsia. 2014 Aug;55(8):1255-63. doi: 10.1111/epi.12713. Epub 2014 Jul 3.

Blood-brain barrier dysfunction can contribute to pharmacoresistance of seizures.

Salar S1, Maslarova A, Lippmann K, Nichtweiss J, Weissberg I, Sheintuch L, Kunz WS, Shorer Z, Friedman A, Heinemann U.

http://dx.doi.org/10.1111/epi.12713

"My curriculum vitae does not appear in the electronic version of my thesis for reasons of data protection."

PUBLICATIONS

Published

1. The differential participation of pyramidal cells in generating spontaneous sharp-wave ripples in the mouse subiculum in vitro.
Maslarova A, Lippmann K, **Salar S**, Rösler A, Heinemann U. Neurobiol Learn Mem. 2015 Aug 28; 125: 113-119

2. In vitro seizure-like events and changes in ionic concentration.
Antonio LL, Angamo EA, Anderson ML, Gabriel S, Klaft ZJ, **Salar S**, Sandow N, Heinemann J. Neurosci Methods. 2015 Aug;21. pii: S0165-0270(15)00305-2

3. Blood-brain barrier dysfunction can contribute to pharmacoresistance of seizures. **Salar S**, Maslarova A, Lippmann K, Nichtweiss J, Weissberg I, Sheintuch L, Kunz WS, Shorer Z, Friedman A, Heinemann U. Epilepsia. 2014 Aug;55(8):1255-63.

4. Increased susceptibility to acetylcholine in the entorhinal cortex of pilocarpine-treated rats involves alterations in KCNQ channels.
Maslarova A, **Salar S**, Lapilover E, Friedman A, Veh RW, Heinemann U. Neurobiol Dis. 2013 Aug;56:14-24.

5. Peri-infarct blood-brain barrier dysfunction facilitates induction of spreading depolarization associated with epileptiform discharges.
Lapilover EG, Lippmann K, **Salar S**, Maslarova A, Dreier JP, Heinemann U, Friedman A. Neurobiol Dis. 2012 Dec;48(3):495-506.

6. Four novel and two recurrent NHLRC1 (EPM2B) and EPM2A gene mutations leading to Lafora disease in six Turkish families.
Salar S, Yeni N, Gündüz A, Güler A, Gökçay A, Velioğlu S, Gündoğdu A, Caglayan HS. Epilepsy Res. 2012 Feb;98(2-3):273-6.

7. Atorvastatin has cardiac safety at intensive cholesterol-reducing protocols for long term, yet its cancer-treatment doses with chemotherapy may cause cardiomyopathy even under coenzyme-Q10 protection.
Caner M, Sonmez B, Kurnaz O, Aldemir C, **Salar S**, Altug T, Bilir A, Altinoz MA. Cell Biochem Funct. 2007 Jul-Aug;25(4):463-72.

8. The effects of tamoxifen on radiation-induced pulmonary fibrosis in Wistar albino rats: results of an experimental study.
Bese NS, Umay C, Yildirim S, Ilvan S, Dirican A, **Salar S**, Altug T, Ober A. Breast. 2006 Jun;15(3):456-60.

In Review

1. A1 Receptor - mediated suppresion of carbamazepine-resistant seizure-like events in human neocortical slices.
Klaft ZJ, Hollnagel JO, **Salar S**, Caliskan G, Schulz S, Horn P, Schneider U, Koch A, Gerevic Z, Gabriel S and Heinemann U.

2. Intraventricular albumin application affects synaptic plasticity in area CA1 of rat hippocampal slices.
Salar S, Lapilover E, Müller J, Hollnagel JO, Lippmann K, Friedman A and Heinemann U.

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