Impairment of insulin signalling pathway in Alzheimer´s disease

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Abstract: The neurodegenerative disorder Alzheimer's disease (AD) is the cause of approximately 60% of the world's 35 million patients suffering from dementia. Current research focuses here are on association with other diseases such as diabetes type 2 (T2DM), possible genetic markers, specific signal transduction pathways within the brain and potential protein modification, because the pathogenesis and etiology of AD are still not fully understood. Specifically association of T2DM with AD came to the focus with the so-called "Rotterdam study" in 1999, indicating that T2DM doubles the risk of developing AD. In the meantime, it is known that the prevalence rate in patients with T2DM is 30%. Drugs commonly used in the treatment of T2DM such as peroxisome proliferator-activated receptors gamma (PPAR) agonists show improvement of the cognitive abilities in patients with early stage of dementia, with potential therapeutically relevance. Therefore it is important not only to investigate a link between these diseases, but also to investigate the insulin signaling pathway in the brain of AD patients. In order to investigate this complex issue in more details and demonstrate additional links between T2DM and AD, the present study used several basic biological methods to clarify the question: "Is impaired insulin signaling pathway within the brain crucial for the development of AD?" from several points of view. The methods used in this work have been i) an analysis of single nucleotide (SNP) polymorphism of the insulin-degrading enzyme gene (IDE) in relation to risk of AD and / or of T2DM, ii) post-mortem histochemical studies of brain tissue of patients with only AD, with AD combined with T2DM and with only T2DM compared with an age-matched control group, and iii.) investigations of neurochemical pathways and gene/protein expression changes of a human cell culture as a consequences of amyloid (A) treatment. After analysis of the IDE SNP polymorphism in the selected VITA (Vienna Trans Danube Aging) cohort disease-specific effects were discovered. The upstream polymorphism (IDE2) was found to influence AD risk in a protective manner, while the downstream polymorphism (IDE7) modified the T2DM risk. Based on the SNP results, the presented study delineate the model that IDE promoter and 3 untranslated region/-downstream variation can have different effects on IDE expression, maybe a relevant endophenotype with disorder-specific effects on AD and T2DM susceptibility. Furthermore, the human post-mortem studies could show that both AD as well as T2DM patients had a significantly lower density of the insulin receptor (IR) in the hippocampus, whereas a significantly increased density of inactive phosphorylated PPAR has been found and this persisted even in patients with both diseases. Summarizing the histological study, it was possible to reveal common histological features of AD and T2DM, but no direct connection between the two diseases. Although AD is nowadays not only characterized by amyloid-containing plaque deposits and by the hyperphosphorylation of tau protein, the excessive A 42 presence in the brains of AD patients is still playing a key role. Up to date it is still not entirely clear which physical form of A 42 is responsible for the development of AD. The present work investigated, what impact has the state of aggregation of A 42 on genes and proteins of the insulin signaling pathway and the amyloid cascade. It could be shown that the oligomeric variant enhanced specifically the gene and protein expression of glycogen synthase kinase (GSK) 3 and also the enzyme activity was significantly increased, but has in turn strongly inhibited the IR gene and protein expression. Additionally, the effect of A 42 on monoamine oxidase B (MAO-B) was examined. An effect of both aggregated forms of A 42 had on enzyme activity was discovered. However, the fibrillar variants led to significantly increased activity of MAO-B while the oligomeric variants inhibited the enzyme activity. Several previous studies have demonstrated the involvement of increased MAO-B activity in AD, but the present work provides for the first time a direct

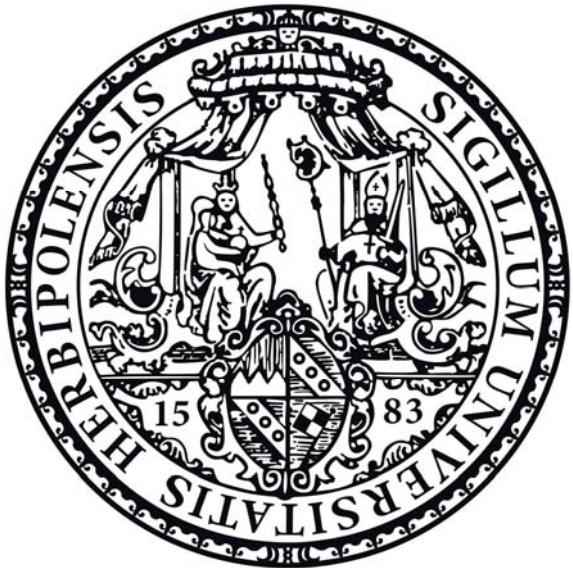
link between the states of aggregation of A 42 to enzyme activity. Finally the results of the presented thesis can be summarized to following conclusion: Although AD and T2DM sharing some degrees of common features, still there is a lack of direct association, and therefore the diseases must be considered more independent rather than linked. But the impaired cerebral insulin signaling pathway seems to be another manifested hallmark of AD.

Für meine geliebten Eltern

Rosi und Dieter Bartl

Impairment of insulin signaling pathway in Alzheimer's disease

Beeinträchtigung des Insulinsignalweges bei Alzheimer Demenz



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Altern ist ein hochinteressanter Vorgang:

Man denkt und denkt und denkt –

plötzlich kann man sich an nichts mehr erinnern.

(Ephraim Kishon, Israelischer Schriftsteller)

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ABBREVATION INDEX

A
ACTB = actin beta
AD = Alzheimer's disease
AIC = Akaike Information Criterion
AID = APP intracellular domain
AKT = protein kinase B
ANOVA = analysis of variance
Apo = apolipoprotein
APP = amyloid precursor protein
Aβ = amyloid beta

B
BACE = β secretase
BBB = blood brain barrier
BMI = body mass index
BS = blocking solution
BSA = bovine serum albumin

C
°C = celsius
Ca = Calcium
CERAD = Consortium to Establish a Registry for AD
cDNA = copy DNA
CO_2 = carbon dioxide

D
DMEM = dulbecco's modified eagle's medium
DNA = deoxyribonucleic acid

E
ELISA = enzyme linked immunsorbent assay
ECL = enhanced chemiluminescence

F
FAD = familial AD

G
g = gramm
GAPDH = glyceraldehyde 3-phosphate dehydrogenase
GSK = glycogen synthase kinase

H
H_2O_2 = hydrogen peroxide
HCL = hydrochlorid acid
HRP = horse redish peroxidase
HWE = Hardy Weinberg equilibrium

I
icv = intracerebroventricular
IDE = insulin degrading enzyme
IGF = insulin growth factor
IL = interleukin
IR = insulin receptor
IRS = insulin receptor substrate

K
KCL = potassium chloride
kD = kilo Dalton

L
l = liter
LOAD = late onset AD

M
MAO = monoamin oxidase
ml = millilitre
mM = millimolar

N
nAChR = nicotin actelycholin receptors
NFT = neurofibrillary tangles
NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
nm = nanometer
NMDAR = glutamate receptors
NSE = neurospecific enolase

O
OS = oxidative stress

P
PCR = polymerase chain reaction
PI3K = phosphoinositide 3-kinases
PPAR = peroxisome proliferator-activated receptors
PPIA = peptidylprolyl isomerase A
PS = presenilin

Q
QRT-PCR = real time reverse transcriptase PCR

R
RNA = ribonucleic acid
RPL13A = ribosomal protein L13a
ROS = reactive oxidative species

S
sAPPα =soluble N-terminal APP fragment-α APP
SD = standard deviation
SNP = single nucleotide polymorphism
STZ = streptozotocin

T
T1DM = type 1 diabetes mellitus
T2DM = type 2 diabetes mellitus
TBS = tris buffered saline
TDZ = thiazolidinediones
TNF = tumor necrosis factor
TOMM = a transporter of proteins across the mitochondrial membrane

U
UTR = untranslated region

V
VITA = Vienna Transdanube Aging

W
WB = western blot

ZUSAMMENFASSUNG

Die neurodegenerative Erkrankung Alzheimer Demenz (AD) ist für etwa 60% der weltweit 35 Millionen Demenz Patienten ursächlich. Die aktuelle Forschung konzentriert sich hierbei auf Assoziationen mit anderen Erkrankungen wie Diabetes Typ 2 (T2DM), potentielle genetische Marker, spezifische Signaltransduktionswege im Gehirn und mögliche Modifizierung von Proteinen, da weder die Pathogenese noch die Ätiologie von AD vollständig geklärt ist. Im Jahr 1999 rückte durch die so genannte "Rotterdam-Studie" eine mögliche Verbindung zwischen T2DM und AD in den besonderen Fokus der Wissenschaft, da die Studie darauf hinweist, dass T2DM das Risiko eine AD zu entwickeln verdoppeln kann. In der Zwischenzeit ist bekannt, dass die Prävalenz an einer AD zu erkranken bei Patienten mit T2DM 30% beträgt. Zusätzlich zeigten Medikamente, die häufig zur Behandlung von T2DM eingesetzt werden, wie PPARγ (Peroxisom-Proliferator-aktivierte Rezeptoren gamma) Agonisten, eine Verbesserung der kognitiven Leistung bei Patienten mit einem frühen Stadium der AD. Daher ist es wichtig, nicht nur eine mögliche Verbindung zwischen diesen Krankheiten zu untersuchen, sondern auch die Insulin-Signalwege im Gehirn von AD Patienten näher zu betrachten. Um dieses komplexe Thema in weiteren Details zu untersuchen und zusätzliche Verbindungen zwischen T2DM und AD aufzuzeigen, verwendet die vorliegende Studie mehrere biologische Grundlagenmethoden, um die Frage zu klären: "Ist ein beeinträchtigter zerebraler Insulin-Signalweg entscheidend für die Entwicklung einer AD?"

Die in dieser Arbeit verwendete Methoden waren i) eine Analyse von Einzel-Nukleotid-Polymorphismen (SNP) des *Insulin-abbauende Enzym* (*IDE*) Gens in Bezug auf das Risiko eine AD und/oder T2DM zu entwickeln; ii) post-mortem histochemische Untersuchungen des Gehirngewebes von Patienten mit nur AD, mit AD und T2DM, und mit nur T2DM verglichen mit einer altersangepassten Kontrollgruppe; und iii) Untersuchungen neurobiologischer Signalwege und Gen-/Protein-Expressions Veränderung einer humanen Neuroblastoma Zelllinie nach Behandlung mit Amyloid β (Aβ) Peptiden.

Nach der Analyse der *IDE*-SNPs in der ausgewählten VITA (*Vienna Transdanube Aging*) Kohorte wurden krankheitsspezifische Effekte entdeckt. Der *Upstream*-Polymorphismus (IDE2) minderte das Risiko an einer AD zu erkranken, während der *downstream* gelegene Polymorphismus (IDE7) das Risiko T2DM zu bekommen, erhöhte. Basierend auf den SNP Ergebnissen, beschreibt die vorliegende Studie ein Modell, das Variationen innerhalb des *IDE* Promotors und/oder in untranslatierten Regionen unterschiedliche Auswirkungen auf die *IDE*

Expression haben können und somit potentiell Auswirkungen auf die Entwicklung von AD und T2DM haben können.

Darüber hinaus konnte die menschliche post-mortem Studie zeigen, dass sowohl AD als auch T2DM Patienten eine signifikant geringere Dichte der Insulin-Rezeptoren (IR) im Hippokampus hatten, während eine signifikant erhöhte Dichte von inaktiven phosphorylierten PPARγ bei allen Patientengruppen detektiert werden konnte. Die vorliegende post-mortem Studie konnte zwar gemeinsame histologische Merkmale von AD und T2DM aufzeigen, jedoch keine direkte Verbindung der beiden Erkrankungen nachweisen.

Obwohl AD heutzutage nicht mehr nur noch durch die Amyloid-haltigen Plaqueablagerungen und durch die hyperphosphorylierten Tau Proteine gekennzeichnet ist, spielt das übermäßige Vorhandensein von $A\beta_{42}$ in den Gehirnregionen von AD Patienten eine entscheidende Schlüsselrolle. Bis dato ist es immer noch nicht vollständig geklärt, welche physikalische Form von $A\beta_{42}$ verantwortlich für eine Entwicklung von AD ist. Die vorliegende Arbeit untersuchte, welche Auswirkungen die Aggregatszustände von $A\beta_{42}$ auf Gene und Proteine des Insulin-Signalweges und auf die Amyloid-Kaskade haben. Es konnte gezeigt werden, dass die oligomere Variante von $A\beta_{42}$ speziell die Gen- und Proteinexpression von Glykogen-Synthase Kinase (GSK) 3β als auch ihre Enzymaktivität deutlich erhöht hatte, jedoch im Gegenzug die IR Gen- und Proteinexpression stark gehemmt hatte. Zusätzlich wurde die Wirkung von $A\beta_{42}$ auf die Monoamin Oxidase-B (MAO-B) untersucht. Es wurde ein Effekt beider untersuchten aggregierten Formen von $A\beta_{42}$ auf die Enzymaktivität entdeckt. Jedoch führte hier die fibrilläre Variante zu einer deutlich erhöhten Aktivität von MAO-B, während die oligomere Variante die Enzymaktivität inhibiert. Frühere Studien konnten bereits eine Beteiligung von erhöhter MAO-B-Aktivität in AD nachweisen, aber die vorliegende Arbeit zeigt erstmals eine direkte Verbindung zwischen den Aggregatzuständen von $A\beta_{42}$ auf die Enzymaktivität auf.

Abschließend können die Ergebnisse der vorliegenden Arbeit zu folgenden Schlussfolgerungen zusammengefasst werden:

Obwohl AD und T2DM bis zu einem gewissen Grad gemeinsame Merkmale aufzeigen, fehlt es an einer direkten Verbindung, und somit sollten die Krankheiten weiterhin eher unabhängig als miteinander verbunden betrachtet werden. Jedoch scheint die Beeinträchtigung des zerebralen Insulin Signalweges ein weiteres gefestigtes Merkmal von AD zu sein.

SUMMARY

The neurodegenerative disorder Alzheimer's disease (AD) is the cause of approximately 60% of the world's 35 million patients suffering from dementia. Current research focuses here are on association with other diseases such as diabetes type 2 (T2DM), possible genetic markers, specific signal transduction pathways within the brain and potential protein modification, because the pathogenesis and etiology of AD are still not fully understood. Specifically association of T2DM with AD came to the focus with the so-called "Rotterdam study" in 1999, indicating that T2DM doubles the risk of developing AD. In the meantime, it is known that the prevalence rate in patients with T2DM is 30%. Drugs commonly used in the treatment of T2DM such as peroxisome proliferator-activated receptors gamma (PPARγ) agonists show improvement of the cognitive abilities in patients with early stage of dementia, with potential therapeutically relevance. Therefore it is important not only to investigate a link between these diseases, but also to investigate the insulin signaling pathway in the brain of AD patients. In order to investigate this complex issue in more details and demonstrate additional links between T2DM and AD, the present study used several basic biological methods to clarify the question: "Is impaired insulin signaling pathway within the brain crucial for the development of AD?" from several points of view. The methods used in this work have been i) an analysis of single nucleotide (SNP) polymorphism of the *insulin-degrading enzyme* gene (*IDE*) in relation to risk of AD and / or of T2DM, ii) post-mortem histochemical studies of brain tissue of patients with only AD, with AD combined with T2DM and with only T2DM compared with an age-matched control group, and iii.) investigations of neurochemical pathways and gene/protein expression changes of a human cell culture as a consequences of amyloid β (Aβ) treatment.

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Furthermore, the human post-mortem studies could show that both AD as well as T2DM patients had a significantly lower density of the insulin receptor (IR) in the hippocampus, whereas a significantly increased density of inactive phosphorylated PPARγ has been found

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