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Prevention of Cognitive Loss by Using Thiamine Plus Either Fluoxetine or Pioglitazone

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Abstract

The notion that a single event might be causative has hampered the search for either a preventative or cure for disturbed cognition. The cellular composition of the brain comprises five cell types: Neurons, microglia, astrocytes, oligodendrocytes and endothelial cells. Successful treatment, whether for prevention or cure of Alzheimer's Dementia (AD), must address all cell types These cell types may interact with each other to form a complex system, with interdependence, synergy and adaptability; once that system has changed so as to create the emergence of Alzheimer's Dementia (AD), it may be very difficult and perhaps impossible, to move it backwards to an earlier, pre-AD state. Therefore, prevention rather than cure should be the goal. Each of three available drugs acts to benefit all five cell types: Fluoxetine, pioglitazone and thiamine. This article describes the actions of each. Two of the drugs given together might be a powerful prophylactic against cognitive loss in persons at risk: Thiamine plus fluoxetine, thiamine plus pioglitazone or fluoxetine plus pioglitazone. Their potential benefit should be tested in a clinical trial.

Keywords: Alzheimer's dementia; Interdependence; Pioglitazone; Synergy

Introduction

The notion that a single event might be causative has hampered the search for either a preventative or cure for disturbed cognition. The cellular composition of the brain comprises five cell types: neurons, microglia, astrocytes, oligodendrocytes and endothelial cells. Knowing that these cell types may interact with each other to form a complex system, with interdependence, synergy and adaptability should make it obvious that any rational therapy must address all five cell types. It should be equally obvious that once that system has changed in such a way as to create the emergence of Alzheimer's Dementia (AD), it may be very difficult and perhaps impossible, to move it backwards to an earlier, pre-AD state. Therefore, prevention rather than cure should be the goal.

To that end, three drugs will be reviewed because there is an extensive literature regarding the benefits conferred by each drug to all five cell types: Fluoxetine, a Serotonin uptake Inhibitor (SSRI); and pioglitazone, which is a thiazolidinedione and Peroxisome Proliferator Activated Receptor gamma (PPARγ) agonist. The third drug, thiamine, will be reviewed but in briefer detail because a previous article provides extensive details [1]. Alone, any one of these five drugs could be prophylactic against cognitive loss; but a dual combination of fluoxetine plus thiamine, pioglitazone plus thiamine or fluoxetine plus pioglitazone, might form a powerful combination for prevention of AD. Before describing the beneficial effects of these drugs, some preliminary general comments are provided concerning the functions and interactions of the five.

Microglia

General comments: In the CNS, activated microglia may have a pro-inflammatory M_1 phenotype or an anti-inflammatory M_2 phenotype. M_1 microglia produce cytokines and chemokines (IL-1β, IL-6, IL-12, TNF-α, CCL-2), express NADPH oxidase and generate reactive oxygen and nitrogen species; M_2 microglia produce anti-inflammatory cytokines (IL-10, TGF-β), growth factors (IGF-1, FGF, CSF-1) and neurotrophic growth factors [2].

There are several conflicting reports concerning the role of microglia in AD, the likely reason being the need to distinguish between proinflammatory and anti-inflammatory microglia. A skewed M_1 activation over M_2 has been related to disease progression in AD [3]. That has also been the case in a mouse model of Parkinson's disease, where microglia have a predominantly M_1 phenotype; treatment with rosiglitazone modulated microglia polarization, and boosted the M_2 over the M1 phenotype [4].

Astrocytes

General comments: The high importance of astrocytes is shown by fact that they account for approximately half of the volume of the adult mammalian brain; that they provide the primary structural and trophic support for neurons; that >99% of the cerebrovascular surface is ensheathed by astrocyte processes; and that each astrocyte supports the functions of 3 or 4 neurons, since processes from a single astrocyte can envelop approximately 140,000 synapses and each cortical neuron has approximately 38,000 synapses [5-6]. The end feet of astrocyte processes, which contain Aquaporin 4, also unsheathe cerebral capillaries; for that reason, astrocytes regulate water permeability and therefore, the transport of drugs across the Blood Brain Barrier (BBB). Aquaporin 4 must regulate that ensheathment, because Aquaporin 4

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knockout mice had ~60% less coverage of cerebral capillaries by astrocytic processes, one result of which was decreased efficacy of fluoxetine [7]. Other important functions of astrocytes are their uptake of the high levels of extracellular potassium that are created from neural activity; their regulation of Ca^{2+} signaling and excitatory neurotransmitters and their delivery of energy to neurons *via* the astrocyte neuron lactate shuttle. In AD, morphological modifications of astrocytes alter K^{+} neurovascular regulation, affecting cerebral blood flow [8]. Astrocyte numbers in the dentate gyrus were more reduced in Braak stages 3-4 than in stages 0-2, which emphasizes their importance for cognition [9].

Oligodendrocytes

General comments: Mature oligodendrocytes perform myelination of naked axons; if their numbers are decreased, e.g. from impaired maturation of Oligodendrocyte Precursor Cells (OPC) so that myelination becomes inadequate, then neural tracts suffer and cognition may be disturbed. Such decrease may occur, e.g. from impaired maturation of Oligodendrocyte Precursor Cells (OPC). Neural Stem Cells (NSC) express the NG-2 proteoglycan marker; OPC express phenotypic markers such as Platelet Derived Growth Factor (PDGF); myelinating mature oligodendrocytes express markers like Myelin Oligodendrocyte Glycoprotein (MOG) and both OPCs and mature oligodendrocytes share markers such as OLIG-2 because mature oligodendrocytes express all three types of glutamate receptors-AMPA, kainate and NMDA they are subject to excitotoxic death.

Neurons

General comments: The recognition of the bidirectional communication between neurons and astrocytes at the synapse led to the concept of the “tripartite synapse” in which the astrocyte, together with the pre and postsynaptic neuronal compartments, is a functional component of the synapse [10]. AD patients with severe tau pathology had a decreased number of newly generated neurons in their dentate gyrus [9].

Endothelial cells

General comments: In all affected areas of the brain in AD, string vessels, that are the remnants of capillary injury, are increased and vascular density is reduced; the cell density of endothelial cells from lithium-treated rodents was higher than those from untreated ones ($p<0.01$) and electron microscopy of the endothelial cells from those treated with lithium, showed normal endothelium whereas those that were untreated had abnormalities such as enlarged auto- phagosomes and dilated ER [11].

Literature Review

The first requisite for formation of a network is that some or all of the cell-types are present together

There is some evidence that the five cell types may form a mutually interactive network. One report showed concurrence of four of the cell-types, microglia, astrocytes, neurons and endothelial cells [12]. Two reports each showed the appearance of three cell types: 1) Endothelial cells, microglia and oligodendrocytes [13]; and 2) Oligodendrocytes, neurons, and astrocytes [10]. Between them, those two reports encompassed all five of the cell types. Many other reports saw two cell types that, between them, also encompassed all five of the cell types [14-19]. While the various reports do not demonstrate an actual network, nevertheless they show that certain circumstances, usually the introduction of a therapy, may engender the joint appearance of the cell types which is the first requisite for their interaction. Nutma actually spoke of the cross talk between astrocytes and oligodendrocytes, explaining that their communication results from direct, cell to cell contact as well as *via* secreted cytokines, chemokines, exosomes and signaling molecules [18]. If a network were to form and advance to a point causing the emergence of AD, there might be difficulty in forcing reversion to a former state. Aside from the example provided by Nutma, extensive evidence exists to show that three drugs, fluoxetine, pioglitazone and thiamine, each affects the five cell types in a way that might interrupt such a network, so that those drugs, either alone or together in a dual combination, could be prophylactic against the occurrence of cognitive loss. The following provides the supporting data which derive from cell cultures, rodent studies and human studies.

Effects of fluoxetine on microglia, astrocytes, oligodendrocytes, neurons and brain endothelial cells

Fluoxetine: A recent meta-analysis examined 14 randomized, controlled trials of Selective Serotonin uptake Inhibitors (SSRI) of which 5 trials used fluoxetine [20]. The 14 studies, involved 550 receiving SSRI and 541 receiving placebo for 8-12 weeks. All participants had a standardized diagnosis of dementia that was restricted to AD and vascular dementia. Cognitive function was assessed by MMSE, demonstrating a beneficial effect of SSRI with a mean difference in scores of 0.84 (P=.002). That difference was NS (P=.58) for the 9 studies not using fluoxetine, but for the 5 studies that used fluoxetine in 158 subjects and placebo in 157, the mean difference was 1.16 (P=.002) favoring fluoxetine.

In the following, data are described from studies based upon cell culture, studies in rodents, and studies in humans; primarily, the studies are mentioned that provide not only the direction of effect upon the particular cell-type but also indicate the mechanism for the effect that was discerned.

Fluoxetine: Cell and Rodent Studies

Astrocytes

Cell studies: Kinoshita et al. showed that fluoxetine increased Brain-Derived Neurotrophic Factor (BDNF); the mechanism being ATP exocytosis from astrocytes *via* Vesicular Nucleotide Transporter (VNUT); and the released ATP and its metabolite adenosine, act on P2Y11 and adenosine A2b receptors that are both expressed by astrocytes [21]. Allaman et al also showed upregulated BDNF, as well as Vascular Endothelial Growth Factor (VEGF) [22]. Genes for BDNF and its receptors are rapidly induced by fluoxetine in cultured astrocytes; and fluoxetine activates the Extracellular signal regulated protein Kinase (Erk) and p38 Mitogen Associated Protein Kinase (MAPK) cascades [23]. Cortical astrocytes from APP/PS1 AD model mice induced synaptotoxicity and also reduction of both dendritic complexity and axonal branching of hippocampal neurons [19]. High levels of soluble β-Amyloid (Aβ) produced by those astrocytes were significantly inhibited by fluoxetine, thus ameliorating neurotoxicity and improving behavioral performance. Finally, Shu et al.

demonstrated that fluoxetine treatment promoted mycophagy and auto phagosome formation, thus increasing clearance of any astrocytes having damaged mitochondria [24].

Microglia

Cell studies: Chung et al. found that in microglial cultures, fluoxetine inhibited expression of proinflammatory cytokines and of inducible nitric oxide synthase; and it attenuated microglial NADPH oxidase activation, production of reactive oxygen species/reactive nitrogen species with consequent oxidative damage [25]. Likewise, Zhang et al. saw significant inhibition by fluoxetine of LPS induced activation of microglia and the subsequent release of multiple pro-inflammatory and cytotoxic factors including tumor necrosis factor-α, interleukin-1β, nitric oxide and reactive oxygen species [26]. Lee et al. also saw significant inhibition by fluoxetine of the microglial activation that had been induced by spinal cord injury [14]. Concordant with decreased expression of proinflammatory cytokines, fluoxetine down regulated M_1 activation and up-regulated M_2 activation in both microglial primary cells and in derivative cell lines [27]. It also decreased the release of glutamate and d-serine from LPS activated microglia, producing an increase in the survival of co-cultured cortical neurons that had been deprived of glucose and oxygen [28].

Oligodendroglia

Cell studies: Fluoxetine inhibited expression of pro-Nerve Growth Factor (pro-NGF), which mediates oligodendrocyte cell death through the p75 neurotrophic receptor; in addition, it attenuated activation of the RAS homolog gene family, and decreased the levels of phosphorylated c-Jun and activated caspase-3 [14]. Those combined effects reduced the death of oligodendrocytes and also, therefore, the resulting decrease of Myelin Basic Protein (MBP) and myelin loss. De Leeuw et al. used Embryonic Stem Cells (ESC) to show that 4/6 oligodendrocyte markers were up-regulated, 2/6 >6-fold, by fluoxetine [29].

Oligodendroglia

Rodent studies: In rats, fluoxetine injections increased the numbers of OPC by 63-80% in the pre-frontal cortex [30]. Chao et al. found a reduced percentage of oligodendrocyte lineage cells displaying the senescence phenotype (CDKN2A/p16INK4a) in the hippocampus of APP/PS1 mice that had been treated with fluoxetine and gave evidence that this delayed senescence was achieved by reducing activation of Glycogen Synthase Kinase (GSK) [31].

Neurons

Cell studies: The rate of differentiation by human Embryonic Stem Cells (hESC) into neuronal precursors was higher in fluoxetine treated cells than in control cells [32]; and Czeh et al. demonstrated that the inhibitory effect of stress upon neurogenesis in the dentate gyrus was counteracted by fluoxetine [30]. Using cultures of midbrain dopaminergic neurons, Zhang et al found that fluoxetine protected against LPS-induced neuronal damage, as shown by attenuated decreases in (3H) DA uptake and in the number of tyrosine hydroxylase positive neurons [26].

Neurons

Rodent studies: In rats, Kodama et al. saw that after fluoxetine administration the majority of newborn cells in their dentate gyrus were neurons [15]. Others confirmed this in middle aged APP/PS1 mice, and also found that fluoxetine alleviated their impaired spatial learning [33]. Perhaps surprisingly, the same investigative group saw no benefit from fluoxetine on the decreased volume and length of myelinated fibers of mice that had been subject to chronic stress [34]. Stanisavljevic et al. used immunohistochemical detection of c-Fos protein expression, to detect activated neuronal circuits in rats subjected to chronic isolation stress; fluoxetine increased activation in several brain areas but only in the striatum was it increased significantly more than in controls [35]. Shan et al. showed that the protection by fluoxetine against neuronal apoptosis that had been induced by IL-1β, required the down-regulation of the p38-p53 pathway [36]. In rats, Bianchi et al used high-performance liquid chromatography and fluorescence detection to quantify alpha-tubulin isoforms [37]. The neuron-specific delta-2 tubulin was increased by chronic fluoxetine; it is likely that the result of such cytoskeletal changes would be reflected by improvement in neural tracts which then might benefit cognition.

Despite the above positive studies, there are occasional ones that did not show neuronal benefit from fluoxetine.

Endothelial Cell Studies

Endothelial cells

Rodent studies: Fluoxetine administration resulted in ~20% of newborn cells becoming differentiated into endothelial cells in rats' prelimbic cortex [15]. Following experimental cerebral artery thrombosis, Ofek et al. saw that infusion of cerebral arterioles with therapeutically effective doses of fluoxetine, produced an increase of vasodilatation by 1.2 to 1.6 fold and this was suppressible by antagonists of muscarinic and nitric oxide signaling [38]. The vasodilatation might not have been from a benefit to endothelial cells, because fluoxetine also decreases tone in arteriolar smooth muscle [39]; however, Hu et al. found that 4-6 weeks after occlusion of the Middle Cerebral Artery (MCA), rats given fluoxetine had up-regulation of the Hypoxia Inducible Factor-1α (HIF-1α)-Netrin/VEGF cascade leading to cerebral micro-angiogenesis due to proliferating endothelial cells, as shown by PCNA-positive cells expressing CD-31 [40].

Fluoxetine

Human studies: Only three studies of fluoxetine administered to humans provide data relevant to the current discussion. First, in a study by Liu et al, 50 patients with vascular dementia were randomly allocated to receive fluoxetine 20 mg/day or no fluoxetine for 12 weeks [41]. At baseline, MMSE scores for patients and controls were 15.1 and 15.7 respectively, and at week 12 they were 16.1 and 15.8 (P=.03); also at baseline, serum levels of BDNF (ng/ml) were 21.2 and 21.4, respectively, and at week 12 they were 25.7 and 21.5, respectively (P<.0001). Second, Hoexter et al. randomized 38 treatment-naïve patients with Obsessive Compulsive Disorder (OCD) to treatment for 12 weeks with either fluoxetine or Cognitive Behavior Therapy (CBT); there were 36 controls, matched with the patients for age, gender, socioeconomic status, level of education and handedness [42]. Because of drop-outs or side effects, only 13 subjects in each

group had follow-up scans. At baseline, the OCD patients had smaller gray matter volume in the left putamen, bilateral medial orbitofrontal, and left anterior cingulate cortices than did controls (P<0.05); after treatment, the Gray Matter (GM) volume in the left putamen increased (P<0.012) in fluoxetine treated patients, whereas no significant GM volume changes were observed in CBT treated patients. The authors provide a detailed discussion of several methodological limitations of this study. Third, Mowla et al. randomized 58 non-depressed subjects with Mild Cognitive Impairment (MCI) to take either fluoxetine or placebo for 8 weeks; only 44 subjects completed the trial, mainly because of side effects (10 with fluoxetine and 4 with placebo) [43]. Between baseline and end of study, patients in the fluoxetine group showed improvement in MMSE score, 24.17-27.00 (P<.002) and in WMS-III scores, for immediate memory 8.35-10.82 (P=.006) and for delayed memory 7.10-9.28 (P=.001).

PPARγ: Effects of PPARγ on Microglia, Astrocytes, Oligodendrocytes, Neurons and Brain Endothelial Cells

PPARγ

General comments: PPARγ is a transcription factor that governs the expression of genes such as those involved in production of Nerve Growth Factor (NGF) and BDNF and of those involved with insulin sensitivity. PPARγ has two isoforms of which PPARγ1 is expressed in neurons and astrocytes. There are different transcriptional outcomes depending on the kinases involved: for example, Ser112 phosphorylation decreases PPARγ activity if induced by MAPK but increases it if induced by Cyclin-Dependent Kinases (CDK) 7 and CDK-9 and phosphorylation at S273 mediated by CDK-5, leads to reduced insulin sensitivity [44]. The PPAR receptor is in the nucleus, where it binds to DNA as a heterodimer in complex with its co-receptor, the 9-cis Retinoic acid Receptor (RXR) [45]. Thiazolidinediones (TZDs) such as pioglitazone, rosiglitazone and ciglitazone, are high affinity agonists for PPARγ.

In several studies, 50-70% of the rodents that were treated with pioglitazone showed benefits: Saunders et al listed 15 rodent studies in which 11 showed benefits that included learning and/or memory in 6/11, reduced Aβ1-42 and/or reduced amyloid plaques in 5/11 and improved LTP in 3/11 [46]. Human studies also showed improvement from pioglitazone in ~70% but no improvement in ~30%; this shows why pioglitazone administration in the context of this article, should be partnered with a second drug for supplemental efficacy.

PPARγ: Cell and Rodent Studies

Astrocytes cell studies

PPARγ action on astrocytes cell-based studies: The Janus-Kinase (JAK) and Signal Transducer Activator of Transcription (STAT) signaling pathways control various cell functions. JAK activation phosphorylates STAT-3 in astrocytes and this phosphorylation links to mitochondrial damage, apoptosis, neuroinflammation and genetic mutations. PPARγ prevents phosphorylation of JAK-STAT in astrocytes, and thereby rescues many dysfunctions, including those producing neuronal dysfunction. The relevance of dosage is illustrated by the effects of ciglitazone, which was cytoprotective for astrocytes at 10 μm but cytotoxic at 20 μm [47]. Curcumin is a PPARγ, agonist and reversed the decreased expression of PPARγ receptor in astrocytes that had been exposed to Aβ25-35 [48]. It is, parenthetically, interesting that turmeric, which contains curcumin, is widely used in India, where the age-adjusted prevalence of AD has been low in several reports. However, a study comparing 91 brains from cases with no history of dementia, from Mumbai, India (age 60+ years; mean age 71.1 years) and compared with identically examined, age-matched samples obtained in New York, showed similar numbers with amyloid plaques and neurofibrillary tangles, suggesting no benefit from use of curcumin [49].

PPARγ action on microglia

Cell based studies: Bernardo et al. reported that PPARγ was constitutively expressed by rats' microglia; that the expression was down-regulated during microglial activation by LPS; that the presence of the PPARγ natural ligand 15d-PGJ2 counteracted that down-regulation and those PPARγ agonists inhibit LPS/IFN-γ induced production of TNF-α and iNOS in microglia [50].

PPARs also act to negatively-regulate gene expression of proinflammatory genes by antagonizing the activities of transcription factors such as members of the Nuclear Factor-κB (NF-κB) and Activator Protein-1 (AP-1) families: the mechanism that underlies the ability of PPARs to interfere with the activities of these transcription factors has been termed trans-repression [51]. Related to this, rosiglitazone administered to a mouse model of Parkinson's disease, up-regulated CD206, which is a marker of M-2 microglia [4]. Finally, demonstrating microglial involvement at a very early stage of the disease pathogenesis, Boza-Serrano et al. found JAK/STAT, p38 MAPK, and interleukin pathways affected in microglial cells before the appearance of amyloid plaques [52].

PPARγ action on oligodendrocytes

Cell based studies: Cultured NSCs expressed detectable levels of oligodendrocyte progenitor-specific NG2 proteoglycan that increased significantly after the addition of ciglitazone [10]. Pioglitazone rescued demyelination caused by anti-MOG autoantibody-induced demyelination, by reducing heat shock responses and downregulating TNF-α [53].

PPARγ action on oligodendrocytes

Rodent studies: Oligodendrocyte lineage cells have receptors for Interleukin-4 (IL-4). Experiments with mice having knockout for either IL-4 or PPARγ, showed that the promotion of OPC differentiation into mature oligodendrocytes by IL-4 was mediated by PPARγ [54]; another study showed that curcumin increased the level of PPAR by 2.25-fold and promoted the maturation of OPC [55].

PPARγ action on neurons

Cell based studies: The neurogenic differentiation gene, NeyroD-1, was increased 3.8-fold by ciglitazone [10]. Pioglitazone protected cultured cortical neurons and axons against nitric oxide-induced toxicity and the neurons showed a significant increase in the expression of PPAR gamma [56]. Likewise, the neurotoxic effect of KCl when added to cerebellar granular cells was prevented by the presence of ciglitazone [57].

PPARγ action on cerebral endothelial cells: Transmission electron microscopy of the whole brains of young mice transgenic for AD, showed abnormal cerebral micro vessels before the

histopathology of AD appeared, indicating that the microvasculature has an early role in pathogenesis [58]. Fibroblast Growth Factor 21 (FGF-21) is an angiogenic molecule; Huang et al. used human brain microvascular endothelial cells to demonstrate that angiogenesis by FGF-21 is achieved *via* activation of PPARγ; an inhibitor of PPARγ combined with FGF-21, significantly reduced the angiogenic activity of FGF-21, whereas activation of PPARγ by rosiglitazone, combined with FGF-21, effected similar angiogenesis as did FGF-21 treatment alone [59].

PPAR*γ* regulates endothelial cells at a variety of stages, including their proliferation; this was shown as dose dependent, since cultured endothelial cells treated with pioglitazone 10-8 M for 5 days showed increased DNA synthesis but a higher dose (10-5 M) suppressed DNA synthesis [60].

PPARγ

Human studies: The studies in humans are more numerous for PPARγ than for fluoxetine, because PPARγ is anti-diabetic; and diabetes, that affects ~10% of the population, is a risk factor for AD. There are approximately similar numbers of studies showing benefit to cognition as those showing no benefit. Following are those showing benefit.

Tseng matched pioglitazone users and the comparison cohort, for comorbidities including hypertension, dyslipidemia, ischemic heart disease, and peripheral arterial disease, Parkinson 's disease, statin use and other glycemic control agents [61]. Average age of each group was 57.7 years; follow-up was for 2 years. Each group, never users and ever users, of pioglitazone had 11,011 subjects; numbers of cases with incident dementia were 123 for never users and 91 for ever users. For pioglitazone use >19.6 months, the hazard ratio for dementia was 0.716 (P=.016) for ever users versus 1.0 for never users. Heneka et al. used observational data from 2004-2010, to analyze the association of pioglitazone and incidence of dementia in a study of 145,928 subjects aged ≥ 60 years who, at baseline, were free of dementia and insulin-dependent diabetes mellitus. Pioglitazone use for >2 years was associated with a lower Relative Risk (RR) for dementia in diabetics relative to non-diabetics RR 0.53 (P=0.029); use for <2 years did not lower the risk of dementia [62]. Consistent results were reported by Miller et al from 142,328 veterans, 98% white males of mean age 66 years, mostly with type 2 diabetes, receiving care from 1999 through 2004, who had initiated either pioglitazone or rosiglitazone, or insulin, without having had prior prescriptions for those medications, and without having had a recorded diagnosis of AD during the previous ≥ 2 years [63]. Patients were followed from the time of drug initiation and monitored for a first diagnosis of AD. The hazard ratio for subsequent AD for the 74,525 who initiated either pioglitazone or rosiglitazone, as compared with the 67,803 who initiated insulin, was 0.81. There was not a non-diabetic comparator group. Liu et al. performed a meta-analysis of nine studies that included none of the above, four of which compared pioglitazone with placebo in 133 subjects with comorbid diabetes, and showed significant benefit for pioglitazone users in the ADAS-Cog score, with mean difference -3.39, (P<0.00001) [64]. Interestingly, neither in this dataset nor in the above-mentioned report by Heneka et al [62], did rosiglitazone lower the dementia risk, which is why in the present article references are largely restricted to the use of pioglitazone.

Thiamine

Cell and rodent studies regarding astrocytes, microglia, oligodendrocytes, neurons and endothelial cells: As indicated earlier, another article [1] lists, in detail, the benefits from thiamine and its potential use in preventing cognitive loss; therefore, only a very brief description is provided here. The importance of thiamine for human cognition was emphasized by results of studies reported by Measelle, infants whose mothers received the highest supplementation dose (10 mg/day) performed the highest on some cognitive assessment tests; breast milk thiamine content at 2 weeks of age, prior to the supplementation of the mothers, was highly predictive of infants' cognitive scores up to 12 months of age [65]. Many other reports of the benefits following thiamine administration are based on studies made in rodents with thiamine deficiency and in humans with Wernicke Encephalopathy who have severe thiamine deficiency. Thiamine Diphosphate (TDP), the active metabolite, is a cofactor in the pyruvate dehydrogenase complex, the α-Ketoglutarate Dehydrogenase Complex (KGDHC), the branched chain α-keto acid dehydrogenase complex, the pentose phosphate pathway (cytosolic transketolase) and in the α-oxidation of phytanic acid (2-hydroxyacyl-CoA lyase); in a thiamine-deficient state, these enzymes limit the supply to and the cycling of the Krebs cycle, resulting in decreased ATP synthesis, oxidative damage and cell death [66]. Those disturbances in thiamine deficiency create a metabolic acidosis. The following very brief description will show that thiamine, certainly when deficient and probably when not clearly so, may benefit all of the cell types under discussion.

Thiamine

Studies in rodents and humans: Astrocytes, microglia, oligodendrocytes neurons and endothelial cells. In experimental thiamine deficiency in rodents and in Wernicke Encephalopathy in humans, there are increases of microglial activity, decreases of astrocyte number and a selective neuronal cell loss. Treatment with thiamine reverses the astrocytic and microglial changes and prevents further neuronal loss [67]. Astrocytes are major regulators of glutamatergic neurotransmission because, in order to prevent excitotoxic cell death, they maintain extracellular glutamate at a low level by using several glutamate transporters to remove glutamate from the synaptic cleft. In rats made deficient in thiamine, Hazell, found a 71% and 51% decrease in levels of the astrocytic glutamate transporters GLT-1 and GLAST, respectively and in both thiamine-deficient rats and in the frontal cortex of patients with Wernicke-Korsakoff encephalopathy, other astrocytic glutamate transporters, EEA-1 and EEA-2, were reduced by 62% and 71%, respectively [68].

Endothelial cells are involved: during thiamine deficiency, indicators of oxidative stress including heme oxygenase-1 (HO-1;8), superoxide dismutase, ferritin and reactive iron, accumulate in microglia, while the nitration products peroxynitrite and nitro tyrosine and the lipid peroxidation product 4-hydroxynonenal, all increase and affect neurons of vulnerable regions, particularly the thalamus; one mechanism for this is an increased production of nitric oxide synthase by endothelial cells, causing pathological levels of nitric oxide [69]. Thus, cultures of brain endothelial cells that had been made deficient in thiamine by addition of pyrimethamine, showed increased glucose consumption and lactate production and increased endothelial cell permeability [70]. KGDHC activity is decreased in AD brains and also in thiamine deficiency. A possible explanation is that there are high levels of the oxidant generating enzyme myeloperoxidase, which was

increased in brains of both AD and the murine APP/PS1 model of AD and both had enhanced adhesion of neutrophils to small vessels; those vessels contained extracellular myeloperoxidase, presumably derived from attached neutrophils, which would affect endothelial cells and contribute to oxidation stress in thiamine deficiency [71].

Oligodendrocytes are also involved: the genes for the surface markers of oligodendrocytes include those for ligands Olig-1, Olig-2, MBP, MAG and MOG; Chatterton et al. reported that chronic thiamine deficiency caused reduced expression of those above genes, while thiamine administration produced their significant recovery [72].

Discussion

Reduced numbers or function of five cell-types, *i.e.,* neurons, microglia, astrocytes, oligodendrocytes, and endothelial cells, are together responsible for the emergence of cognitive loss and eventual occurrence of AD. It would seem a priori that concurrent prevention of those reductions in all five cell types could be highly effective in preventing cognitive loss. Shown here, each of these five cell types gains benefit from exposure to each of three drugs, thiamine, fluoxetine and pioglitazone. Elsewhere, it has been suggested that supplementary thiamine might alone prevent cognitive loss [1]: it is clear that its combination with either fluoxetine or pioglitazone could be a very powerful prophylactic in persons at risk.

The hypothesis presented in this article is that individuals with diabetes, which affects ~10% of the population, or prediabetes that affects another 30%, would benefit from thiamine plus pioglitazone and those with a history of depression, which affects 3.7% of the population but in the SARS-CoviD-2 pandemic affects 25% [72,73], would benefit from thiamine plus fluoxetine. The others might benefit from pioglitazone plus fluoxetine.

Conclusion

This hypothesis is easily testable by a clinical trial that would randomize participants aged >70, to receive either the chosen pair of drugs or placebo and follow them for up to 5 years. The required number of participants would be based on a power of 80% to achieve a reduction of AD by 50%. The trial, by arrangement, could terminate in <5 years when occurrence of AD in those on active drug is <50% of the diagnosis in those on placebo.

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