Anti-inflammatory substances – a new therapeutic option in Alzheimer's disease

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Among several pathogenetic elements underlying Alzheimer's disease (AD), a brain-specific inflammatory response has recently attracted attention as a cause of neurodegeneration and progressive cognitive decline. Markers of inflammation in AD are activated microglial cells, synthesis of cytokines, acute-phase proteins and complement proteins in areas of brain destruction. Epidemiological studies point to a reduced risk of AD among users of anti-inflammatory drugs. Influencing inflammatory parameters has become the focus of several new treatment strategies and a clinical trial with indomethacin shows promising results. The results from current clinical trials with steroidal and non-steroidal anti-inflammatory drugs will be available in the near future.

Age itself is the primary 'risk factor' of AD. The risk of developing AD increases with age (Table 1). Dementia affects 4–8% of the worldwide population older than 65 years and AD accounts for more than 50% of all cases where dementia is diagnosed. In developed countries, AD is one of the major disabling diseases, with an enormous impact on the budgets of the social security and health systems. In the USA, more than two million people are affected; in Germany, 10% of the population is older than 65 years and from a total population of 80 million, approximately 0.6 million suffer from AD (Ref. 1).

Effects of anti-inflammatory substances on the risk of developing AD

Several studies have investigated the correlation between the use of anti-inflammatory substances and the risk of developing AD. Of 15 epidemiological studies, 14 showed that a reduced risk of developing AD is associated with the previous use of steroidal or non-steroidal anti-inflammatory drugs (NSAIDs)[2]. A reduced risk was also found in a study with homozygous twins. This argues against genetic differences as a potential common predisposition to anti-inflammatory treatment and to a decreased risk of developing AD. Two recent epidemiological studies from Europe and the US confirm the finding that the risk of developing AD is reduced by 50% in users of anti-inflammatory drugs[3,4].

Genetic factors

Cases of AD that are caused exclusively by genetic factors are extremely rare. Worldwide, only about 180 families have been described so far where an autosomal dominant form of AD is clearly linked to different mutations in three different genes[5]. These genes code for the presenilin-1 protein on chromosome 14, for the presenilin-2 protein on chromosome 1 and for the amyloid precursor protein on chromosome 21, and all three genes seem to interfere with the processing of the amyloid precursor protein. Except for a few patients with mutations in the gene encoding

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Table 1. Field studies on the prevalence and incidence of dementia

Age (years)	Prevalence (%)	Incidence (%)
65–69	2	0.1–0.8
75–79	5–7	1.0–3.8
85–89	16–23	2.0–7.0

presenilin-2, all individuals carrying these mutations develop AD before the age of 65 (Ref. 6). The effect of anti-inflammatory treatment in these rare, genetically caused variants of AD is not known.

The apolipoprotein-E4 allele of the three different allelic forms of apolipoprotein-E is associated with a higher risk of developing AD. The risk of carriers that are homozygous for the apolipoprotein-E4 alleles is more than threefold greater than for individuals without an apolipoprotein-E4 allele. However, the apolipoprotein-E4 allele is neither a necessary nor a determining factor for the development of AD, thus, genetic analysis of the apolipoprotein-E polymorphism is not useful as a diagnostic marker in AD (Refs 7,8). The pathogenetic pathway influenced by the apolipoprotein-E4 allele is not known, and hypotheses that link apolipoprotein-E polymorphism with either amyloid metabolism or tau pathology are in debate[9,10]. Interestingly, the synthesis of apolipoprotein-E and amyloid-precursor protein is elevated under inflammatory conditions, and the presence of apolipoprotein-E in amyloid plaques has been correlated with the activation of microglial cells[11,12].

Neuropathology of AD

The major neuropathological hallmarks of AD are amyloid plaques, neurofibrillary degenerations and loss of synapses, the latter being most closely correlated with the cognitive decline[13]. Potential functional connections between the loss of synapses, amyloid deposition and neurofibrillary degeneration are unclear.

The major component of amyloid plaques is the βA4 protein, a fragment derived from the amyloid precursor protein (APP). *In vitro* studies of the neurotoxicity of βA4 show inconsistent effects depending on the cell culture system[14]. High-density cultures of primary neurons are usually quite resistant to βA4 toxicity. In many neuronal cell culture systems a delayed cell death is measureable after 48 h of incubation with 1–100 μM βA4 (Ref. 15). The degree of toxicity of amyloidogenic peptides derived from the βA4 sequence seems to depend on the aggregation of these peptides into fibrils of 7–9 nm. The dynamics of aggregation and the degree of toxicity is also extremely heterogenous between different peptides derived from the full 1–42 amino acid sequence of βA4. For example, the hydrophobic, slow-aggregating β-peptide 29–35 needs several days of preincubation before maximal toxicity is observed. By contrast, the β-peptide containing the amino acids 25–35 displays maximal toxicity immediately after dissolution[14]. Several lines of evidence support the hypothesis that, like other amyloidogenic peptides, βA4 in cell culture induces a necrotic cell death by a common pathway involving the generation of free radicals[16,17]. *In vivo* injection of βA4 is not associated with major neurodegeneration[18]. Although the loss of synapses is not markedly accentuated within amyloid deposits, pathological processes initiated in amyloid deposits might alter distant synaptic cell contacts[19,20].

Another neuropathological hallmark of AD is the formation of tangles in the neuronal cell soma and neuritic degenerations of neuronal processes. Both intracellular lesions consist of paired helical filaments (PHF), which disturb the neuronal cytoskeleton. PHF in the neuronal processes and PHF of perinuclear tangles consist of hyperphosphorylated tau proteins. The microtubuli of the cytoskeleton are stabilized by tau, which belongs, therefore, to the so-called microtubule-associated proteins[21,22]. The binding of tau to micobuli is diminished by phosphorylation and restored by dephosphorylation[23]. The formation of tangles starts in the neuronal somata in the transentorhinal cortex, expanding into the hippocampus and further into the neocortex. Braak and colleagues demonstrated that the cognive decline in dementia parallels the different stages of tangle formation[24]. If tangles are restricted to the transentorhinal cortex, dementia is usually absent; however, neocortical tangles are always associated with a moderate to severe dementia[25].

Neuritic degenerations of neuronal cell processes are found throughout the cortex in AD and are not restricted to amyloid plaques. Diffuse amyloid plaques usually contain only a few degenerated neurites[26]; by contrast, a corona of degenerative neurites are always present in primitive and classic amyloid plaques[27]. In hippocampal cell cultures, βA4 can induce hyperphosphorylation of tau[28]. However, neurofibrillary degenerations are not restricted to amyloid plaques and are widely distributed through the brain in AD. The intracellular accumulation of PHF and the associated disruption of the normal cytoskeleton is accompanied by a shrinkage of the dendritic tree. Therefore, neurofibrillary degeneration might facilitate the loss of synapses.

Inflammatory mechanisms

Activation of microglia

The activation of microglial cells in AD has been found consistently in neuropathological investigations. Amyloid

plaques are not only found in AD but are also present in non-demented controls. By contrast with AD, amyloid plaques in non-demented controls are less frequently neuritic amyloid plaques, being mainly early diffuse amyloid plaques without major neuritic degenerations. Mackenzie and colleagues showed that the proportion of neuritic amyloid plaques do not accumulate in a fixed ratio to the amount of diffuse amyloid plaques in non-demented controls[29]. They further showed that the number of detectable microglial cells in diffuse and neuritic amyloid plaques is higher in AD than in the sporadic diffuse amyloid plaques in non-demented controls[30]. They concluded that the activation of microglial cells in the diffuse amyloid plaque might be an important step in the transformation of diffuse amyloid plaques into neuritic amyloid plaques and the development of dementia in AD.

The activation of microglial cells in amyloid plaques has been further investigated with respect to morphological changes of microglial cells and the microglial expression of the major histocompatibility complex class II (MHC II). In AD, microglial cells in diffuse amyloid plaques show an enlarged or phagocytotic morphology[31]. The microglial expression of MHC II is found in the diffuse amyloid plaques in AD but not in the resting microglial cells in the diffuse amyloid plaques in age-matched controls[32]; this supports the hypothesis that the activation of microglial cells is elevated in AD. Activated microglial cells are already found in early diffuse amyloid plaques, which tend to develop into neuritic amyloid plaques. Activation of microglial cells might, therefore, stimulate the formation of neuropathology in AD.

Cultured microglia can be activated *in vitro* by the amyloid precursor protein and βA4 (Ref. 33). In microglial cells, βA4 activates mitogen-activated protein kinases, leading to the phosphorylation of the transcription factor cAMP-response element binding protein (CREB)[34]. Furthermore, βA4 stimulates the production of superoxide by activation of protein tyrosin kinases[35]. However, diffuse amyloid plaques contain resting ramified microglial cells, with no signs of activation in non-demented controls[30,32]. More than 80% of all activated microglial cells in AD are not associated with amyloid plaques[31]. Therefore, the presence of βA4 is neither a necessary nor a sufficient explanation for the activation of microglial cells in AD. However, the presence of βA4 might facilitate microglial activation and the additive effect of βA4 and activated microglial cells might accelerate neuritic degeneration in the diffuse amyloid plaque. *In vitro* amyloid components can stimulate microglial cells to exert toxic effects on cultured neurons[36]; however, neuronal cell loss is only slightly increased in amyloid plaques[37,38].

In a recent animal study it has been shown that indomethacin can attenuate the activation of microglia induced by intraventricullar infusion of βA4 (Ref. 39). Whether the reduction of microglia activation by indomethacin is caused by inhibition of prostaglandin production, interaction with βA4 or other mechanisms is unclear. In addition to the amyloid precursor protein and βA4, the complement component C1q, another potent activator of microglia, is also found in amyloid plaques[40,41]. Interestingly, recent investigations also show a widespread activation of microglia outside amyloid plaques in AD, confirming early observations made in Alzheimer's laboratory at the beginning of the 20th century. High numbers of activated microglia cells are not only found in amyloid plaques but also in hippocampal areas without amyloid deposition[31,42]. The activation of microglia in the hippocampus shows a much better correlation with the extent of tangle formation along the entorhinal–hippocampal pathway than with amyloid deposition[43]. Therefore, microglial activation does not only follow amyloid deposition in AD. In conclusion, the activation of microglia is a fourth component in the progression of AD, in addition to loss of synapses, amyloid plaques and neurofibrillary tangles. Microglia activation might be partly dependent on the other three types of lesion but also might be caused by other, unknown processes (Fig. 1).

Synthesis of cytokines and acute-phase proteins

The cytokines interleukin-1α (IL-1α) and interleukin-6 (IL-6) have been found in amyloid plaques[44,45]. Activated microglial cells express membrane-bound IL-1α (Fig. 2). The production of βA4 and synthesis of cytokines are processes that might stimulate each other[46]. IL-1α and IL-6 have been found in early diffuse amyloid plaques and mature neuritic amyloid plaques, and both cytokines might contribute to the progression of neuropathology[47,48]. The elevated production of IL-6 in the brains of AD patients exceeds the detection limits of biochemical analysis after extraction of brain tissue[49]; therefore, presumably significant amounts of IL-6 are present in the brains of AD patients. Activated astrocytes, as found in astrogliosis, are a potent souce of IL-6 in the brain[50]. However, IL-6 is one of the most potent cytokines to stimulate astrogliosis in the brain[51]. Astrogliosis in AD has been discussed as a final consequence either of amyloid deposition or the loss of synapses[52,53]. However, the gliotic remodelling in AD is a dynamic, ongoing process, as demonstrated by the persistent elevation of the mRNA for glial fibrillary acidic protein (GFAP), a known marker for astrogliotic remodelling[54].

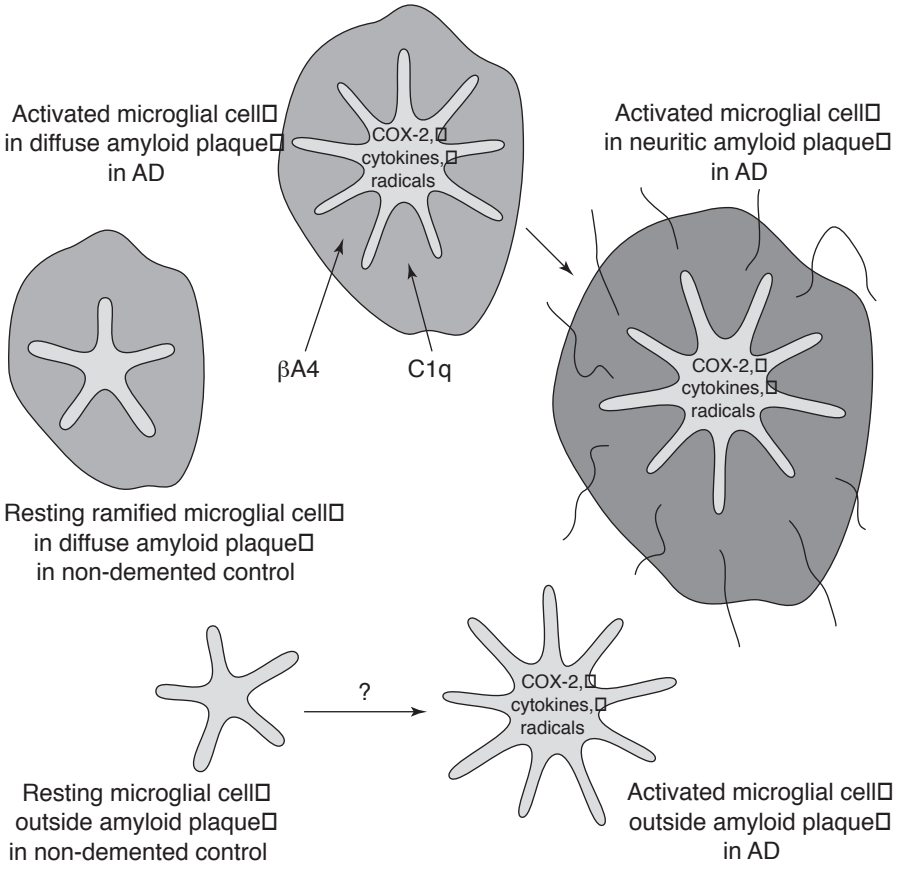

Figure 1. *Activation of microglial cells in Alzheimer's disease (AD). Diffuse amyloid plaques are present in non-demented, age-matched controls and in AD. In the brains of non-demented controls, resting ramified microglial cells are found outside and inside diffuse amyloid plaques. In AD, microglial cells outside and inside diffuse amyloid plaques are activated and produce cytokines, prostaglandins and oxygen radicals. βA4 and the complement component C1q are probably involved in the activation of microglial cells in amyloid plaques. Activated microglial cells stimulate the progression of the plaque development from diffuse to neuritic amyloid plaques. The lack of microglial activation in diffuse amyloid plaques in non-demented age-matched controls and the activation of microglial cells outside amyloid plaques point to other factors beside amyloid in the activation of microglia. COX-2, cyclooxygenase-2.*

Transgenic animals expressing IL-6 under the control of a brain-specific promotor show severe neurological deficits[55], such as alterations in dendritic arborization, a loss of cholinergic innervation of the hippocampus, alterations in long-term potentiation and memory deficits[56–58]. In accordance with the induction of astrogliosis after intracerebral injection of IL-6, these transgenic animals also show a marked astrogliosis[59]. IL-6 is a potent inducer of acute-phase protein synthesis. In correspondence with this known function, acute-phase proteins, such as α_1-antichymotrypsin and C-reactive protein, are found in amyloid plaques and elevated concentrations of these acute-phase proteins have been measured in brain extracts of AD patients[45,49,60,61]. The acute-phase protein α_1-antichymotrypsin has been implicated in the formation of amyloid fibrils[62]. Furthermore, several acute-phase proteins are potent protease inhibitors. Therefore, acute-phase proteins might interfere with the extracellular degradation of amyloid and the growth of amyloid plaques.

Expression of cyclooxygenase-2 in AD

Cyclooxygenase-2 (COX-2) is expressed constitutively in neurons in the brains of AD patients and age-matched controls[63]. In neurons the gene encoding COX-2 is regulated rapidly by synaptic activity and belongs to the immediate early genes. The function of COX-2 in neurons is unclear. The enzymatic generation of oxygen radicals as a byproduct of COX-2 in neurons might also add to free-radical overload and neurodegeneration. There are diverging reports concerning an elevated expression of COX-2 in neurons in AD (Refs 63,64). Activated microglial cells and IL-1-stimulated astroglial cells can also synthesize COX-2 (Refs 65,66). In animal models, hypoxia is a potent stimulus for COX-2 expression in microglial cells, astrocytes and neurons. In human autopsy studies, a high rate of COX-2 mRNA degradation is found, as expected for the mRNA of an immediate early gene. A well-controlled post-mortem study indicates a higher variability of COX-2 mRNA in the brains of AD patients, compared with age-matched controls[67]. Furthermore, a correlation between the presence of the transcription factor nuclear factor

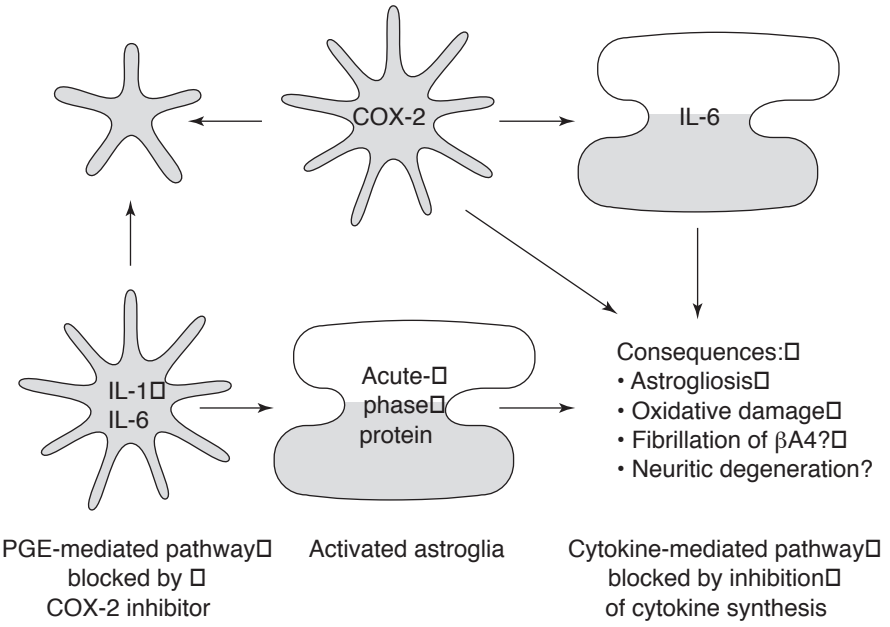

Figure 2. *The activation of microglial cells can be amplified by cytokines or prostaglandins, which can further stimulate astroglial cells to synthesis IL-6 and acute-phase proteins. Astrogliosis, an enhanced fibrillation of βA4 and neuritic degenerations, can result from the combined action of cytokines, acute-phase proteins and oxidative radicals in Alzheimer's disease (AD). Anti-inflammatory drugs might block prostaglandin production, cytokine production or both. COX-2, cyclooxygenase-2; IL, interleukin; PGE, prostglandin E.*

κ-binding (NF-κB) in the cell nucleus and the level of COX-2 mRNA is found in brain tissues of AD patients and age-matched controls, suggesting that NF-kB is involved in the induction of COX-2 in the human brain[68]. The activation of NF-kB has previously been shown in neurons surrounding amyloid plaques in AD[69]. Interestingly, NF-kB is not only involved in the induction of COX-2 but also in the induction of IL-6 and α_1-antichymotrypsin[70–72].

COX-2 synthesizes prostaglandin E_2 (PGE_2) and other prostanoids. PGE_2 induces COX-2 in cultured rat microglial cells[73]. Therefore, a kind of autocrine or paracrine amplification of the COX-2 induction in microglial cells, or a spreading of COX-2 expression between different cells, seems to be possible. Interestingly, PGE_2 also induces IL-6 in astrocytotic cells[74]. Besides the activation of PGE_2 receptors, activation of adenosine A_{2A} receptors leads to an increase in COX-2 expression in microglia[72]. Both stimuli lead to an intracellular increase of cAMP, and inhibitors of cAMP formation reduce COX-2 expression. By contrast with peripheral monozytes, cultured rat microglia cells do not synthesize COX-2 in response to IL-1 or IL-6 (Ref. 66). The regulation of COX-2 might, therefore, differ between cells of the CNS and peripheral cells.

Activation of the complement system

C1q is found in mature amyloid plaques and appears to be attached to tangle-bearing and normal neurons[75]; its influence is not known. Binding of C1q to cell membranes might be initiated by βA4 (Ref. 76). Apart from C1q, several other components of the complement system have been found to be attached to neuronal membranes in AD (Ref. 77). Some reports show the full assembly of the complement membrane attack complex in AD brains, but others show a termination of the complement assembly before the complete membrane attack complex is formed[78,79].

Animal models

Results of studies with transgenic animals are difficult to interpret. Several transgenic approaches using the overexpression of the normal *APP* gene do not lead to any neuropathology. However, transgenic expression of mutated forms of the human *APP* gene cause neuropathological changes in some strains of transgenic animals[80,81]. In a transgenic mouse strain expressing the human *APP* gene containing a known pathogenic mutation (V717F), a progressive deposition of βA4 is associated with dystrophic alterations of neurites and gliotic changes[82]. Interestingly, this process is not randomly distributed, but seems to be primarily distributed along the entorhinal–hippocampal pathway, which is the earliest brain area affected by neuropathology in AD (Ref. 83). The implication for the majority of cases suffering from sporadic AD without any mutation in the *APP* gene is unclear. The deposition of βA4 and the development of memory deficits are also shown in transgenic mice carrying a double mutation of the human *APP* gene (K670N, M671L)[81]. An accumulation of activated microglial cells in the area of amyloid deposition has been demonstrated in these animals[84]. Superoxide dismutase and hemoxygenase-1

protein levels are elevated around βA4 deposits and especially in the area of dystrophic neurites[85]. Therefore, activated microglia might play a major role in the progression of neuropathology in this animal model, which seems to be very well suited to the investigation of anti-inflammatory drugs and radical scavengers. The development of neuropathology in this strain of animal is further augmented by crossbreeding with transgenic animals carrying a mutation in the presenilin-1 gene (M146L)[86]. Other strains of transgenic animals with the *APP* gene are being created and could provide further insights into the pathogenetic role of APP and βA4 (Ref. 87).

Therapeutic approaches to inflammation in AD

Inhibition of cyclooxygenase

Despite controversial data concerning the expression of COX-2 in AD, overwhelming epidemiological data confirm a protection against the development of dementia among users of NSAIDs. In a neuropathological study, the activation of microglial cells in the brains of long-term users of NSAIDs was markedly reduced, compared with age-matched controls[88]. Data from comprehensive clinical trials with NSAIDs in AD are not yet available, but a pilot study with indomethacin showed promising results[89]. However, the statistical power of this pilot study, with 28 participants and a duration of six months active treatment, is too weak to draw final conclusions. Also, gastro-intestinal disturbances, a well known side effect of indomethacin, meant that there was a high rate of drop outs. A new selective inhibitor of COX-2 from Searle, celecoxib[90], is currently being tested in an ongoing trial for AD (see Table 2).

Inhibition of IL-6 synthesis

The newly developed NSAID tenidap inhibits not only the enzymatic function of COX-2 but also the synthesis of IL-6. This has been shown especially for human astrocytotic cells, which might be responsible for IL-6 synthesis in AD (Ref. 91). The suppression of astrocytotic IL-6 synthesis has also be shown for tepoxalin, another novel non-steroidal anti-inflammatory drug (B.L. Fiebich *et al*., unpublished). Newly developed NSAIDs with the ability to suppress cytokine and prostaglandin synthesis could be most attractive candidates for the treatment of AD.

Steroidal drugs

In the middle of this year the data from a large, NIH-supported trial with Prednison in AD, conducted by the Alzheimer's Disease Collaborative Study Unit, will be available[92]; to date, no serious side-effects have been reported[93]. The choice of steroids as drugs in AD requires several other considerations besides the ability of steroids to suppress inflammation. Several animal studies suggested that long-term elevation of steroid concentrations can impair memory function[94]. In healthy elderly persons, steroids reduce hippocampal glucose utilization and memory function[95,96]. By contrast with estrogen, corticosterone potentiates the toxic properties of oxygen radicals, glutamate and βA4 in hippocampal cell culture[97]. Although steroids are potent inhibitors of microglial activation, COX-2 induction and pro-inflammatory cytokine synthesis, their influences on neuronal metabolism and cell death might reduce their benefical anti-inflammatory effect on the progression of AD.

Reduction of microglial activation

A reduced microglial activation can be achieved with NSAIDs and steroidal drugs. However, further substances that have not been developed primarily for their anti-inflammatory potential might prove to reduce microglial activation. Propentofyllin, a component from Hoechst-Marrion-Russel, is an inhibitor of the adenosine transporter and cAMP phosphodiesterase but has also major inhibitory effects on the activation of microglial cells[98]. In microglial cells *in vitro*, propentofyllin reduces proliferation and the production of oxygen radicals[99]. In sepsis, propentofyllin has been shown to reduce the production of tumor-necrosis factor and IL-6 (Ref. 100). Its effects on the synthesis of cytokines or COX-2 in brain cells is not well known. Propentofyllin is being tested in clinical trials for the treatment of vascular dementia and AD (Ref. 101).

Table 2. Approaches to reduce inflammatory activation in AD[a]

Drug class	Action	Examples	Remarks
Steroid	Suppression of protein synthesis of COX-2 and cytokines	Prednison	Ongoing clinical trial. Direct negative influence on hippocampal function
NSAID	Inhibition of the enzymatic function of COX-2	Indomethacin, celecoxib	Gastrointestinal side effects
NSAIDs that inhibit cytokine synthesis	Inhibition of the enzymatic function of COX-2, suppression of IL-6 synthesis	Tenidap, tepoxalin	Proteinuria. No extensive experience in older patients

[a]Abbreviations: AD, Alzheimer's disease; COX-2, cyclooxygenase-2; NSAID, non-steroidal anti-inflammatory drug.

Conclusion

Several epidemiological and neuropathological studies document the involvement of inflammatory mechanisms in AD. The imminent results from a large study with steroids in AD will be the first available from several studies investigating anti-inflammatory drugs. NSAIDs, which block COX-2 enzyme activity and reduce cerebral synthesis of cytokines, might be a good choice for further studies. The fact that other substances, not primarily developed to suppress inflammation, can reduce microglial activation via unknown pathways, also warrants investigation of their possible therapeutic value in the treatment of AD.

REFERENCES

1 Förstl, H. (1998) *Alzheimer's Disease – From Basic Research to Clinical Applications* (Gertz, H.J. and Arendt, T., eds), p. 1, Springer, Wien
2 Breitner, J.C.S. (1996) *Neurobiol. Aging* 17, 789–794
3 Stewart, W.F. *et al.* (1997) *Neurology* 48, 626–632
4 Andersen, K. *et al.* (1995) *Neurology* 45, 1441–1445
5 Anonymous (1998) *Neurobiol. Aging* 19, 109–116
6 Hardy, J. (1997) *Trends Neurosci.* 20, 154–159
7 Saunders, A.M. *et al.* (1996) *Lancet* 348, 90–93
8 Breitner, J.C.S. (1996) *Lancet* 347, 1184–1185
9 Roses, A.D. (1996) *Annu. Rev. Med.* 47, 387–400
10 Gutman, C.R. *et al.* (1997) *J. Neurosci.* 17, 6114–6121
11 Saitoh, T. *et al.* (1997) *Gerontology* 43, 109–118
12 Uchihara, T. *et al.* (1995) *Neurosci. Lett.* 195, 5–8
13 Terry, R.D. *et al.* (1991) *Ann. Neurol.* 30, 572–580
14 Iversen, L.L. *et al.* (1995) *Biochem. J.* 311, 1–16
15 Hensley, K. *et al.* (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 3270–3274
16 Behl, C. (1997) *Cell Tissue Res.* 290, 471–480
17 Mattson, M.P. and Rydel, R.E. (1996) *Nature* 382, 674–675
18 Winkler, J. *et al.* (1994) *Neurobiol. Aging* 15, 601–607
19 Masliah, E. *et al.* (1990) *Am. J. Pathol.* 137, 1293–1297
20 Masliah, E. (1995) *Histol. Histopathol.* 10, 509–519
21 Goedert, M. *et al.* (1992) *Neuron* 8, 159–168
22 Goedert, M. *et al.* (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 4051–4055
23 Gong, C.X., Grundke-Iqbal, I. and Iqbal, K. (1994) *Neuroscience* 61, 765–772
24 Braak, H. and Braak, E. (1997) *Neurobiol. Aging* 18, 351–357
25 Bancher, C. *et al.* (1993) *Neurosci. Lett.* 162, 179–182
26 Yamazaki, T. *et al.* (1991) *Acta Neuropathol.* 81, 540–545
27 Wisniewski, H.M. and Terry, R.D. (1973) *Prog. Neuropathol.* 2, 1–26
28 Busciglio, J. *et al.* (1995) *Neuron* 14, 879–888
29 Mackenzie, I.R.A. (1994) *Acta Neuropathol.* 87, 520–525
30 Mackenzie, I.R.A., Hao C.H., Munoz, D.G. (1995) *Neurobiol. Aging* 16, 797–804
31 Sheng, J.G., Mrak, R.E. and Griffin, W.S.T. (1997) *Acta Neuropathol.* 94, 1–5
32 Sasaki, A. *et al.* (1997) *Acta Neuropathol.* 94, 316–322
33 Barger, S.W. and Harmon, A.D. (1997) *Nature* 388, 878–881
34 McDonald, D.R. *et al.* (1998) *J. Neurosci.* 18, 4451–4460
35 McDonald, D.R., Brunden, K.R. and Landreth, G.E. (1997) *J. Neurosci.* 17, 2284–2294
36 Giulian, D. *et al.* (1996) *J. Neurosci.* 16, 6021–6037
37 Sugaya, K., Reeves, M. and McKinney, M. (1997) *Neurochem. Int.* 31, 275–281
38 Lassmann, H. *et al.* (1995) *Acta Neuropathol.* 89, 35–41
39 Netland, E.E. *et al.* (1998) *Neurobiol. Aging* 19, 201–204
40 Rogers, J. *et al.* (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 10016–10020
41 Watson, M.D. *et al.* (1997) *Amyloid Int. J. Exp. Clin. Invest.* 4, 147–156
42 Roe, M.T. *et al.* (1996) *J. Neuropathol. Exp. Neurol.* 55, 366–371
43 DiPatre, P.L. and Gelman, B.B. (1997) *J. Neuropathol. Exp. Neurol.* 56, 143–149
44 Griffin, W.S.T. *et al.* (1995) *J. Neuropathol. Exp. Neurol.* 54, 276–281
45 Strauss, S. *et al.* (1992) *Lab. Invest.* 66, 223–230
46 Del Bo, R. *et al.* (1995) *Neurosci. Lett.* 188, 70–74
47 Mrak, R.E., Sheng, J.G. and Griffin, W.S.T. (1995) *Hum. Pathol.* 26, 816–823
48 Hüll, M. *et al.* (1995) *Acta Neuropathol.* 89, 544–551
49 Wood, J.A. *et al.* (1993) *Brain Res.* 629, 245–252
50 Maimone, D., Guazzi, G.C. and Annunziata, P. (1997) *J. Neurol. Sci.* 146, 59–65
51 Balasingam, V. *et al.* (1994) *J. Neurosci.* 14, 846–856
52 Liu, X.Y., Erikson, C. and Brun, A. (1996) *Dementia* 7, 128–134
53 Salinero, O. *et al.* (1997) *J. Neurosci. Res.* 47, 216–223
54 Le Prince, G. *et al.* (1993) *Neurosci. Lett.* 151, 71–73
55 Campbell, I.L. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 10061–10065
56 Steffenson, S.C., Campbell, I.L. and Henriksen, S.J. (1994) *Brain Res.* 652, 149–153
57 Bellinger, F.P. *et al.* (1995) *Neurosci. Lett.* 198, 95–98
58 Heyser, C.J. *et al.* (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1500–1505
59 Chiang, C.S. *et al.* (1994) *Dev. Neurosci.* 16, 212–221
60 Abraham, C.R., Selkoe, D.J. and Potter, H. (1988) *Cell* 52, 487–501
61 Rozemuller, J.M., Stam, F.C. and Eikelenboom, P. (1990) *Neurosci. Lett.* 119, 75–78
62 Ma, J. *et al.* (1994) *Nature* 372, 92–94
63 O'Banion, M.K., Chang, J.W. and Coleman, P.D. (1996) *Adv. Exp. Med. Biol.* 407, 171–177
64 Oka, A. and Takashima, S. (1997) *Neuroreport* 8, 1161–1164
65 O'Banion, M.K. *et al.* (1996) *J. Neurochem.* 66, 2532–2540
66 Bauer, M.K. *et al.* (1997) *Eur. J. Biochem.* 243, 726–731
67 Lukiw, W.J. and Bazan, N.G. (1997) *J. Neurosci. Res.* 50, 937–945
68 Lukiw, W.J. and Bazan, N.G. (1998) *J. Neurosci. Res.* 53, 583–592
69 Kaltschmidt, B. *et al.* (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 2642–2647
70 Lieb, K. *et al.* (1996) *J. Neurochem.* 66, 1496–1503
71 Lieb, K. *et al.* (1996) *J. Neurochem.* 67, 2039–2044

72 Bauer, M.K.A. *et al.* (1997) *Eur. J. Biochem.* 243, 726–731
73 Minghetti, L. *et al.* (1997) *Eur. J. Neurosci.* 9, 934–940
74 Fiebich, B.L. *et al.* (1997) *J. Neurochem.* 68, 704–709
75 Afagh, A. *et al.* (1996) *Exp. Neurol.* 138, 22–32
76 Valazquez, P. *et al.* (1997) *Nat. Med.* 3, 77–79
77 McGeer, P.L. *et al.* (1989) *Neurosci. Lett.* 107, 341–346
78 Itagaki, S. *et al.* (1994) *Brain Res.* 645, 78–84
79 Veerhuis, R. *et al.* (1996) *Acta Neuropathol.* 91, 53–60
80 Games, D. *et al.* (1995) *Nature* 373, 523–527
81 Hsiao, K. *et al.* (1996) *Science* 274, 99–102
82 Irizarry, M.C. *et al.* (1997) *J. Neurosci.* 17, 7053–7059
83 Su, Y. and Ni, B. (1998) *J. Neurosci. Res.* 53, 177–186
84 Frautschy, S.A. *et al.* (1998) *Am. J. Pathol.* 152, 307–317
85 Pappolla, M.A. *et al.* (1998) *Am. J. Pathol.* 152, 871–877
86 Holcomb, L. *et al.* (1998) *Nat. Med.* 4, 97–100
87 Calhoun, M.E. *et al.* (1998) *Nature* 395, 755–756
88 Mackenzie, I.R. and Munoz, D.G. (1998) *Neurology* 50, 986–990
89 Rogers, J. *et al.* (1993) *Neurology* 43, 1609–1611
90 Penning, T.D. *et al.* (1997) *J. Med. Chem.* 40, 1347–1365
91 Fiebich, B.L. *et al.* (1996) *Neuroreport* 7, 1209–1213
92 Aisen, P.S. and Pasinetti, G.M. (1998) *Drugs Aging* 12, 1–6
93 Aisen, P.S. *et al.* (1996) *Dementia* 7, 201–206
94 McEwen, B.S. and Magarinos, A.M. (1997) *Ann. New York Acad. Sci.* 821, 271–284
95 Lupien, S.J. *et al.* (1997) *J. Clin. Endocrinol. Metab.* 82, 2070–2075
96 De Leon, M.J. *et al.* (1997) *J. Clin. Endocrinol. Metab.* 82, 3251–3259
97 Goodman, Y. *et al.* (1996) *J. Neurochem.* 66, 1836–1844
98 Schubert, P. *et al.* (1997) *Ann. New York Acad. Sci.* 825, 1–10
99 Si, Q.S. *et al.* (1996) *Exp. Neurol.* 137, 345–349
100 Mandi, Y., Farkas, G. and Ocsovszky, I. (1995) *Acta Microbiologica et Immunologica Hungarica* 42, 301–308
101 Kittner, B., Rössner, M. and Rother, M. (1997) *Ann. New York Acad. Sci.* 826, 307–316

In short...

The US Food and Drug Administration (FDA) has advised **Medeva plc** (London, UK) that further information is required before reviewing the licensing application for Hepagene™ as a vaccine against Hepatitis B. Hepagen was accepted for review by the European Agency for the Evaluation of Medicinal Products last October. The FDA is questioning the number of subjects used for safety evaluation and is requesting modifications to the current manufacturing process and further information on the protocols relating to the validation of equipment, processes and systems, and comparative clinical data on final manufacturing lots. Medeva's Chief Executive, Dr Bill Bogie said 'We remain confident of the clinical merits of Hepagene as a vaccine…and will work with the FDA to address the matters they have raised'.

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