MEETING REPORT

Alzheimer's Disease, Cancer and the Search for a Better Aspirin Postgraduate Course at Mount Sinai School of Medicine, New York, NY June 22–23, 2001

Alexander Scriabine

Yale University School of Medicine, New Haven, CT, USA

Key Words: Aging—Alzheimer's disease—Aspirin—Cancer—Cell cycle genes—COX-2—Cyclooxygenases—Inflammation—Neurodegeneration—NSAIDs—Protein kinases.

The meeting was organized and chaired by **Giulio Maria Pasinetti**, Associate Professor of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA. It was attended by ca. 150 physicians and basic medical scientists and consisted of 23 presentations on the effects of non-steroidal antiinflammatory drugs (NSAIDs), primarily cyclooxygenase 2 (COX-2) inhibitors, in Alzheimer's disease (AD) and cancer. The meeting was supported by grants from Merck & Co. and Helsinn Healthcare.

In his introductory remarks **Pasinetti** pointed out that there is substantial evidence that NSAIDs protect from colon cancer. They may also slow the progression of Alzheimer's disease. He expressed hope that this meeting will provide an impetus for further evaluation and eventual use of NSAIDs in the therapy of these diseases. The first lecture, "Building a Better Aspirin, Experimental Approaches and Clinical Implications," was delivered by **D. L. DeWitt** (Michigan State Univ., Lansing, MI, USA). He emphasized the importance of selective inhibition of COX-2 isoenzyme for side effect profile of inhibitors. Minor changes in the amino acid composition of COX-2, as compared to COX-1, increase the binding site pocket. Inhibitors with bulky side groups are more selective for COX-2 because they fit better to the larger binding site pocket. COX-2 inhibitors, unlike COX-1, do not require acidic side group for binding to the isoenzyme. The COX-2 + inhibitor complexes are not easily reversible, whereas binding of the inhibitors to COX-1 is freely reversible. There are also different modes of inhibition: time-dependent and competitive. Selective COX-2 inhibitors inhibit the isoenzyme time-dependently, the degree of inhibition increases with time. The same inhibitors may inhibit COX-1 competitively. At a low dose, aspirin is a selective COX-1 inhibitor. Among new COX-2 inhibitors valdeco-

Address correspondence and reprint requests to: A. Scriabine, M.D., Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06420, USA.

xib (Searle) is more selective for COX-2 than celecoxib (CELEBREX®) or rofecoxib (VIOXX®).

According to **M. J. Thun** (Am. Cancer Soc., Atlanta, GA, USA) the evidence for anti-cancer activity of NSAIDs includes epidemiological studies in colorectal cancer, experimental studies in animals, and randomized clinical studies with sulindac and celecoxib in patients with familial adenomatous polyposis (FAP). Either of these two NSAIDs effectively suppress the growth of adenomatous polyps in patients with FAP. Prolonged use of NSAIDs is associated with a lower risk of adenomatous polyps and/or colorectal cancer. In rats with chemically-induced colorectal cancer NSAIDs are protective, but duration of treatment appears to be more important than dose. The mechanism of action may involve enhancement of apoptosis or inhibition of angiogenesis. COX-2 is widely expressed during angiogenesis and COX-2 inhibitors block release of angiogenic factors. Celecoxib was shown to potently inhibit angiogenesis. Phase III clinical studies with COX-2 inhibitors as anti-cancer drugs are now in progress.

J. C. S. Breitner (Johns Hopkins Univ., Baltimore, MD, USA) spoke about NSAIDs in AD. He briefly reviewed the results of 23 published reports on prevention or treatment of AD with NSAIDs. Most of these reports were retrospective epidemiological studies. Breitner concluded that there is substantial evidence that antiinflammatory drugs delay the onset of AD and that NSAIDs are, in this respect, more effective than steroids. NSAIDs appear to have a preventive effect at doses lower than required to control inflammation. NSAIDs-like preventive effect can also be obtained with H₂-antagonists, antioxidants (vitamins C and E), or hormone replacement therapy (HRT). Acetaminophen has no preventive effect. NSAIDs appear to be effective only if taken for longer than two years. There is no evidence that these drugs have any beneficial effect in Alzheimer's patients, if the therapy is started after or even just before the onset of the disease. There is no evidence that antiinflammatory activity per se is solely responsible for the preventive effect of COX inhibitors, although COX inhibition is probably required for the preventive effect. Antithrombotic effects of COX inhibitors may conceivably contribute to their ability to delay the onset of AD. Since both COX isoenzymes are present in the brain, future clinical studies in the prevention of AD should utilize COX-1 as well as COX-2 inhibitors.

According to **P. S. Aisen** (Georgetown Univ., Washington, D.C., USA) The National Institute of Aging (NIA) is currently conducting clinical studies with NSAIDs on the prevention as well as in the treatment of AD. One study is comparing the effects of rofecoxib, naproxen and placebo in slowing the rate of cognitive decline in AD. This is a one-year long study with 2 months washout period. NIA is also supporting a primary prevention trial that is designed to compare celecoxib with naproxen. The selection of naproxen was based on its availability. The manufacturer of naproxen was willing to provide a free supply of the drug. One clinical study with a COX-2 inhibitor in AD is being conducted by Merck & Co. An adverse interaction between COX and cholinesterase inhibitors, an increase in gastrointestinal toxicity, was recently demonstrated.

Animal studies with COX inhibitors have shown that COX-2 expression in the brain can be correlated with the distribution of plaques and that COX-2 is upregulated in neurodegenerative lesions. "Exitotoxic stress" with kainate increases the expression of COX-2 in the brain. Treatment of transgenic mice with ibuprofen for 6 months reduces amyloid deposits. Cognitive functions in animals can be stabilized by indomethacin.

A beneficial role of antiinflammatory drugs in colon cancer was further discussed by **R. F. Jacoby** (Univ. of Wisconsin, Madison, WI, USA). FAP is a hereditary autosomal

dominant syndrome. Polyps are likely to form adenomas and eventually colon cancer. The etiology of FAP may involve hereditary mutation of tumor suppressor gene. NSAIDs appear to suppress polyposis; sulindac was demonstrated to reduce the number of polyps in FAP; its effect is reversible. Celecoxib (400 mg b.i.d.) is approved for the treatment of FAP. Another type of hereditary colon cancer (HNCC) is not preceded by polyposis, this type is not likely to be prevented by NSAIDs. COX-2 inhibitors are effective in 80% of patients with FAP. Because of their effectiveness and relative safety in FAP they should be preferred to other NSAIDs. Epidemiological and animal data further support the use of COX-2 inhibitors in the treatment of FAP.

G. M. Cole (Univ. of California at Los Angeles, CA, USA) discussed the etiology of AD and the preventive effects of NSAIDs in animal models of AD. One of the animal models is the Upjohn (APP_{SW}) transgenic mouse. Drugs decrease the formation of plaques and decrease the levels of beta amyloid (A β)-associated phosphotyrosine in this model. Chronic treatment of transgenic mice with ibuprofen suppressed astrocytosis, microgliosis, amyloid deposition, IL-1 β , activation of caspase, and soluble A β . Cole believes that NSAIDs prevent microgliosis as well as astrocytosis and that these effects are beneficial in AD. These drugs may, however, produce also oxidative damage. In a search for other approaches to Alzheimer's therapy Cole discovered beneficial effects of curcumin, an antioxidant from curry spice. He evaluated curcumin in another animal model of Alzheimer's disease (rats subjected to intracerebral infusion of A β protein). Curcumin prevented oxidative damage, reduced amyloid deposition and cognitive deficits and restored LTP in these rats. Overall microgliosis was reduced, but microglia were recruited toward plaques. Soluble ApoE was reduced and CREB elevated. Curcumin also reduced total cholesterol levels. The effectiveness of curcumin was confirmed in APP_{SW} mice. Also in this model curcumin suppressed elevated oxidative damage, IL-1 β , soluble A β and amyloid deposits. Cole feels that curcumin has multiple sites of action. He studied the safety of curcumin using turmeric (that contains 1 to 5% curcumin). It produced no side effects in rats at 3500 mg/kg/day for 90 days, but reduced oxidative brain damage at 2000 mg/kg/day for 18 weeks.

S. Gandy (Nathan Kline Institute, Orangeburg, NY, USA) reviewed the role of gonadal hormones in cancer and neurodegeneration. Epidemiological studies indicate that women on hormone replacement therapy have lower incidence of AD. According to Gandy ovariectomy increases A β levels in the brain of guinea pigs and this effect is normalized by 17- β -estradiol. In humans androgen suppression therapy for prostate cancer increases plasma A β levels. The mechanism of A β lowering effects of gonadal hormones is not yet well understood. Estrogens may influence APP processing as well as increase A β clearance.

Curcumin

S. J. Hewett (Univ. of Connecticut Health Center, Farmington, CT, USA) discussed the role of COX-2 in excitotoxic neurodegeneration. Persistent activation of NMDA receptors on neuronal surface can lead to neuronal degeneration. This effect is at least partially me-

diated by calcium and is accompanied by upregulation of neuronal COX-2. NMDA-stimulated production of prostaglandins (Pgs) by neurons is coupled to COX-2. *In vitro*, NMDA-induced neuronal death can be prevented by COX-2 antagonists. By intracerebral administration to mice NMDA can destroy neurons in the CA1 region. Pretreatment with naproxen, 10 to 20 mg/kg i.p. antagonizes these effects of NMDA. Hewett concluded that COX-1 and COX-2 antagonists may produce therapeutic benefit in conditions associated with the increased neuronal excitotoxicity. According to **I. Vincent** (University of Washington, Seattle, WA, USA) it is evident that inappropriate cell division leads to premature neuronal death in patients with AD. Appearance of cell cycle regulators precedes ultrastructural lesions in AD. These regulators initiate neurodegeneration by triggering abortive cell cycles. In AD mitotic activation occurs in cytoplasm instead of nucleus, it leads to abortive mitosis and neuronal death. Vincent speculated that inherited neurodegenerative mutations induce transformation of terminally differentiated neurons, leading to neuronal death. Apoptosis may not be only the only fate of affected neurons. All possible connections between cell cycle pathway and neurodegeneration should be further explored. Drugs that are currently being developed for the treatment of cancer may conceivably be also useful in the treatment of neurodegenerative diseases (See also special issue of *Neurobiology of Aging*, Vol. 21, No. 6, edited by Vincent and Coleman, for further discussion of this topic).

S. Therianos (University of Rochester, Rochester, NY, USA) discussed loss of synapses and shrinking of the dendritic tree in the brain of Alzheimer's patients. He emphasized heterogeneity of neuronal damage in the course of AD. Not all neurons are in the same stage of degeneration. Neurons containing tangles may survive for years. Elucidation of factors determining their survival may help in the search for new approaches to the treatment of AD. He also suggested that synaptic functioning may become suboptimal in AD, even when synapses are structurally intact.

G. Einstein (NIH, Bethesda, MD, USA) outlined the NIH extramural grants review process and problems with the review of multidisciplinary research proposals. Research proposals in the Alzheimer's field are often multidisciplinary, but NIH review process is organized largely along disciplinary lines. It is important that a grant application is submitted to and reviewed by the appropriate Institute and Study Section. True multidisciplinary applications should be marked as such. If an application involves one discipline in preference to others, this discipline should be appropriately identified. The NIH web site should be consulted to properly identify the discipline and the study section that is likely to review a particular grant application. The main criteria in the grant review process are: significance of the proposed research, validity of the approach and degree of scientific innovation.

S. M. Prescott (University of Utah, Salt Lake City, UT, USA) reviewed connections between inflammation and cancer. COX-2 enhances production of prostaglandins leading to inflammation and COX-2 is overexpressed in colon cancer. Inhibition of COX-2 slows the rate of growth of colon cancer. Prescott studied factors controlling the expression of COX-2 mRNA. Phorbol esters increase expression of COX-2 mRNA and are carcinogenic. IL-2 also enhances COX-2 mRNA expression, but with a different time course. COX-2 expression is regulated at the transcriptional and the translational levels. Stable cell lines have a tight control over COX-2 protein expression. Alterations in the binding of several RNA-binding proteins contribute to the defective regulation of COX-2 that may lead to cancer and/or inflammation.

G. M. Pusinetti (Mount Sinai School of Medicine, NY, USA) discussed the role of COX-2 in the regulation of the wingless (WNT) signal transduction pathway in the brain of transgenic mice. As an Alzheimer model, Pusinetti is using triple transgenic mice obtained by crossing APP_{SWE}/PS1-A246E mice with mice that overexpress human COX-2 in neurons. The overexpression of exogenous COX-2 in these mice leads to a three-fold elevation in S⁷⁹⁵ phosphorylated tumor suppressor protein and active caspase 3 immunopositive neurons. These effects reflect cell-cycle activity. Overexpression of hCOX-2 in primary cortico-hippocampal neurons derived from hCOX-2 transgenics enhances A β -induced apoptosis and neuronal death. This effect can be inhibited by flavoperidol, a cell-cycle-dependent kinase inhibitor. Hippocampal COX-2 protein content correlates linearly with the clinical progression of early AD dementia. In transgenic mice an increase in COX-2 expression accelerates plaques formation.

The role of COX-2 in prostate cancer was discussed by **A. C. Levine** (Mount Sinai School of Medicine, NY, USA). She studied the expression of COX-2 in the human prostate gland. This enzyme is strongly expressed in smooth muscle cells of normal and neoplastic prostate. In normal prostate the expression is limited to the basal cell layer, whereas in prostatic inflammation it is expressed also in luminal epithelial cells. COX-2 inhibitors induce apoptosis in prostate cancer cells, prevent upregulation of vascular endothelial growth factor (VEGF), and decrease tumor microvessel density and angiogenesis. According to epidemiological studies men taking NSAIDs regularly have a reduced incidence of prostate cancer. Benign prostatic hypertrophy (BPH) can be reduced or prevented by COX-2 inhibitors. Levine concluded that COX-2 inhibitors are protective and possibly even therapeutic in prostate cancer.

A. J. Danneberg (Cornell Univ. Medical School, New York, NY, USA) reviewed the role of inflammation in cell signalling and cancer. Experimental breast cancer in rats can be prevented by celecoxib or ibuprofen. NSAIDs prevent also experimental head and neck squamous cell carcinoma (HNSCC) in animals. COX-2 is overexpressed in HNSCC. Increased levels of PGE₂, and COX-2 protein as well as increased rates of COX-2 transcription were found in HER-2/neu transformed mammalian cells. Inhibitors of MAPK kinase decreased the levels of COX-2 in these cells. Aberrant MAPK signaling may contribute to overexpression of COX-2 in HNSCC.

T. Arendt (Paul Fletching Institut für Hirnforschung, Leipzig, Germany) proposed that an elevated expression of the cell cycle in AD may lead to neurofibrillary degeneration. According to Arendt a labile state of differentiation in a subset of neurons in the adult brain may render these neurons particularly vulnerable. AD affects preferentially neurons with a high degree of plasticity. Inhibitors of cyclin-dependent kinases (e.g., p16) are strongly expressed in degenerating neurons in AD. These inhibitors may represent a protective mechanism from cell-cycle reentry and apoptosis and may conceivably prevent neurodegeneration in AD.

M. A. Smith (Case Western University, Cleveland, OH, USA) discussed cell cycle abnormalities and abortive oncogenesis in AD. Various cell cycle control elements have been recently associated with the neurodegeneration in AD. Aberrant mitogenic signaling may be involved in the pathogenesis of AD. Smith studied chronology of activation of kinases (ERK, JNK/SAPK, and p38) during progression of AD. In non-demented elderly individuals without any AD pathology either ERK alone or JNK/SAPK alone can be activated. In non-demented cases with limited pathology both, ERK and JNK/SAPK (but not p38), are activated. All three kinases are activated in mild and severe cases of AD. Smith

concluded that MAPK pathways are differentially activated during the course of AD. He suspects that both, oxidative stress and mitotic alterations, are necessary for the progression of AD.

M. Hull (Division of Medicine, University of Leeds, Leeds, UK) discussed the role of COX-2 and antiinflammatory drugs in β -catenin expression and Wnt signaling. In COX-2 positive as well as COX-2 negative human colorectal cancer cells (CRCs) indomethacin induced G1 arrest and apoptosis. N-398, a selective COX-2 inhibitor, induced G1 arrest and apoptosis only in COX-2 positive CRCs. Indomethacin downregulated β -catenin expression and target gene, cyclin D1, in SW 480 and HCT 116 cells. Sulindac has also been reported to decrease β -catenin expression *in vitro*. The effect of indomethacin was associated with complex changes in downstream WNT signaling. It is not clear whether these effects of indomethacin and other NSAIDs are COX-dependent or independent.

NS-398

R. Bhat (AstraZeneca, Huddinge, Sweden) implicated the GSK3 β (a Ser/Thr kinase) signal transduction pathway in neurodegeneration. He reported previously that the GSK3 β pathway plays a critical role in apoptosis mediated by NGF withdrawal in PC12 cells. Bhat demonstrated recently that multiple apoptotic stimuli activate GSK3 β and that this activation is accompanied by phosphorylation on Tyr216. Insulin and lithium, inhibitors of GSK3 β pathway, inhibited cell death. Bhat studied also GSK3 β activation in animal models of neurodegeneration. In rats with middle cerebral artery occlusion (MCAO) he found an increase in GSK3 β phosphorylation in parietal cortex at 3 h after occlusion and reperfusion. In rats, subjected to intrahippocampal injections of NMDA, GSK3 β activity was increased in the CA1 region prior to neuronal degeneration. Bhat concluded that GSK3 β is involved in neuronal degeneration and that inhibition of the GSK3 β pathway might offer a new approach to the therapy of neurodegenerative diseases.

P. C. Salinas (Imperial College of Science, Technology and Medicine, London, UK) spoke about the role of the Wnt signal transduction pathway in the brain. Salinas and associates have recently demonstrated that the Wnt signaling family regulates the formation of central synapses. Wnt-7a decreases axonal extension, increases growth cone size and spreading along the axon and induces clustering of synaptic proteins in developing axons. In transgenic mice Wnt-7a appears to act as a retrograde signal to regulate presynaptic differentiation in cerebellum. The axonal remodeling and protein clustering by Wnt-7a is mediated by inhibition of GSK3 β . Salinas suggested that regulation of GSK3 β activity is central to synaptic remodeling. She is currently investigating the effects of mood stabilizing drugs on GSK3 β activity and axonal remodeling. Salinas suggested further that the WNT-GSK3 β pathway may regulate neuronal plasticity in adult life.

J. Campisi (Lawrence Berkeley National Laboratory, Berkeley, CA, USA) reviewed the role of inflammation in cell senescence. She proposed that cell senescence halts cell division irreversibly and prevents formation of tumors, since tumors arise exclusively from mitotic cells. However, senescent cells accumulate with age and secrete molecules, including inflammatory cytokines, e.g., IL-1, that compromise tissue integrity and function. Accumulation of senescent glial cells may contribute to the inflammatory processes in the brain.

P. Davies (Albert Einstein College of Medicine, New York, NY, USA) discussed shared mechanisms in tau pathology and cell cycle activities and implications for the discovery of a novel target for a better aspirin. In AD aberrant cell cycle mechanisms might influence phosphorylation as well as conformation of tau. cdc2-Like kinase is activated early in the development of AD. In AD tau is phosphorylated at threonine 231. This phosphorylation creates a binding site for Pin1, a proline isomerase involved in mitosis. During the cell cycle Pin1 binds to cdc2 phosphorylated epitopes of proteins and alters their activity by isomerization of ser/thr proline bonds. Pin1 copurifies with helical filaments that accumulate in AD and has a high affinity for these structures. Pin1 may also bind to APP. Davies suggested that in AD tau and APP undergo conformational changes through the action of Pin1. Degenerative changes in the brain of patients with AD may result from inappropriate action of proteins usually associated with cell division.

In her closing remarks **M. Sano** (Columbia Univ., New York, NY, USA) emphasized that NSAIDs may have multiple mechanisms of action. Each of them may be useful in controlling disease and each of them should be further investigated. Ideal treatment should target the early and least symptomatic phase of the disease. Identification of patients most likely to benefit from a particular therapy is useful in conducting clinical trials as well as in directing drug development.