A Combination of Free-B-Ring Flavonoid and Flavan, Anti-Inflammatory Plant Extract Maintains Memory and Speed of Processing in Animal and Human Clinical Models

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Abstract

Over 1200 plant extracts were screened for novel cyclooxygenase (COX) and lipoxygenase (LOX) inhibitors. Two polyphenol extracts derived from the roots of *Scutellaria baicalensis* and heartwoods of *Acacia catechu* containing Free-B-Ring flavonoids and flavans, respectively, were combined into a proprietary blend called UP326. UP326 also induced a down-regulation of cox-2, interleukin-1β (il-1β), tumor necrosis factor-α (tnfα), and interleukin-6 (il-6) gene expression concomitant with a decrease in nuclear factor kappa B (nfκb) mRNA levels in cells exposed to lipopolysaccharide (LPS). UP326 had a high ORAC value of 5,517 μmole TE/g indicating a strong antioxidant capacity. We used a radial arm water maze (RAWM) and contextual fear conditioning (CFC) to assess the effect of UP326 fed to aged Fischer (F344) male rats for changes in memory and learning. In a separate human clinical trial, test subjects administered 300 mg of UP326 per day for 30 days showed marked improvement in speed and accuracy of processing complex information and reduced their standard deviation of performance compared to baseline and the placebo group. This data suggests that UP326 might maintain memory, help sustain speed of processing, and reduce the number or memory errors as we age.

Introduction

By the year 2050, the annual incidence of Alzheimer's disease (AD) is expected to double from 377,000 (1995) to 959,000 (2050) primarily affecting persons born between the years of 1946 and 1964[1,2]. Recent reports have identified correlations between COX-2 expression, general inflammation and the pathogenesis of AD leading to loss in memory and speed of processing[3,4]. The protective effect of non-steroidal anti-inflammatory drugs (NSAIDs) in the pathogenesis of AD is attributed to COX-2 inhibition and the direct prevention of amyloid plaque accumulation in the brain[5]. By suppressing COX-2 production of the pro-inflammatory prostaglandin PGE_2, the surrounding neurons are also spared from the oxidative and inflammatory insult that would be generated by activated microglia[3]. This action eliminates the subsequent microglial generation of cytokines and reactive oxygen species (ROS) that feed the cycle and propagate neurodegeneration[6,7]. Aging and oxidative stress are associated with declines in hippocampal processing of information as demonstrated by the deficits seen in spatial learning, memory formation and the decline in long term potentiation[8,9,10]. The present study analyzed the effects of UP326 supplementation on short-term memory in aged rats and speed as well as accuracy of processing complex information in humans. The findings suggest that improvements might be due to inhibiting cytokine production and improving oxidative status in the brain.

Materials and Methods

UP326 was tested for *in vitro* COX-2 and 5-LOX inhibitory activity using a cleavable, peroxide chromophore included in the assay to visualize the peroxidase activity of the enzymes in presence of arachidonic acid as a cofactor[11,12,13]. Gene expression assays were performed by inducing inflammation in purified peripheal blood monocytes from three subjects with 10 ng/ml LPS for 18-h,

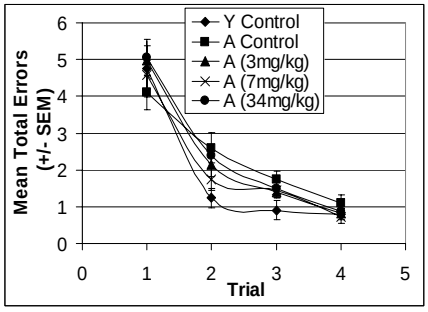
purifying mRNA, and performing quantitative RT-PCR using the ABI Taqman® system with primer/probe sets specific to cox-2, il-1β, tnfα, il-6, and nfκb genes on an ABI 7700 sequence detector. $ORAC_{hydro}$ reflects water-soluble antioxidant capacity. Trolox, a water-soluble Vitamin E analog, was used as the calibration standard and the ORAC result for UP326 was expressed as micromole Trolox equivalent (TE) per gram. F344 male rats (Harlan), age 6 and 18-mo, were initially trained to the same level of proficiency for 3-wk in a RAWM before oral dosing of UP326 in 4 different dose groups (0, 3, 7, or 34 mg/kg, n=12 per group) for 2-mo versus young controls (n=12) fed the NIH31 control diet. The rats were then tested for CFC, another test that measures hippocampal-memory effects while controlling for improved motor function. Rats were placed in a box with an electrified grid also having specific auditory and olfactory cues. Two days after initial training, behavioral immobility (% freezing) was measured under 3 conditions: the original training apparatus, a novel apparatus, and the novel apparatus with the same auditory cue as present during training. In the human study, test subjects (n=43) were administered 300 mg of UP326 per day. A series of computer tasks were performed weekly by the test subjects via the internet to test speed and accuracy in processing complex commands and information and the data compared to the placebo group (n=40).

Results

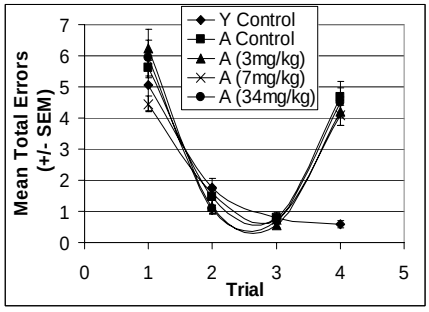
In oxygen sensing assays, UP326 had an IC_{50} of 1.02μM for COX-2 inhibition and an IC_{50} of 3.8μM for 5-LOX inhibition compared to COX-2 IC_{50} of 1.68μM for indomethacin, 278μM for aspirin, 0.2μM for Licofelone™ also with a 5-LOX IC_{50} of 0.2μM[14]. UP326 had an ORAC value of 5,517 vs vitamin C at 5,000 μmole TE/g indicating a high capacity to reduce ROS. UP326 reduced LPS induced inflammatory gene expression by: 63-fold for cox-2, 45-fold for il-1β, 3.3-fold for tnfα, 37-fold for il-6, and 2.2-fold for nfκb. This *in vitro* data was corroborated with *in vivo* efficacy by performing an aged rat study and human clinical trial. After initial training in the RAWM but before administration of UP326, aged rats were able to perform as well as their young cohorts (Figure 1A). But after a 4-h delay was inserted between trials 3 and 4, the aged rats lost all training advantages (Figure 1B). After taking UP326 for 2-mo, all groups showed improved attenuation of age-related memory impairments (Figure 1C).

Figure 1

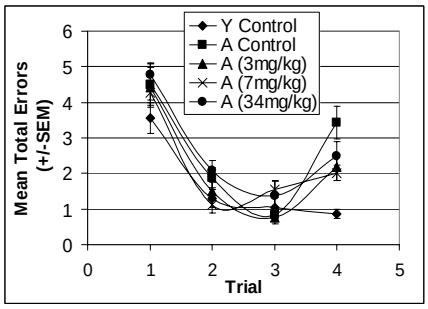
A.



B.

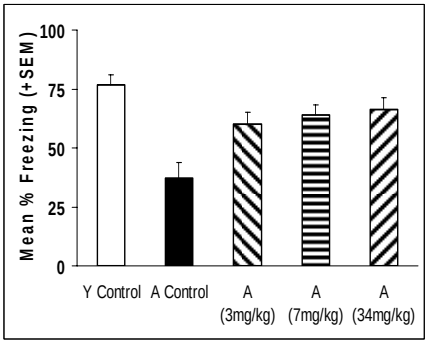


C.



Memory was also tested using CFC conditioning (normal behavioral immobility in an environment previously paired with foot shock) to rule out any anti-inflammatory effects of UP326 on improvements in motor function. CFC testing showed an improvement in normal freezing behavior in aged rats given UP326 for 2-mo approaching that of the young control rats (Figure 2). UP326 also had no effect on a direct measure of nociceptive threshold (data not shown).

Figure 2



Loss of function in human cognition generally occurs slowly, taking a decade or more to appear in standard laboratory exams. Speed of processing, however, can be measured in a relatively short period of time. Test subjects administered computer tasks to test the speed and accuracy of processing of complex information in a 4-wk clinical trial had significant improvement in the speed and accuracy of judging complex information compared to the placebo group (Figure 3).

Figure 3

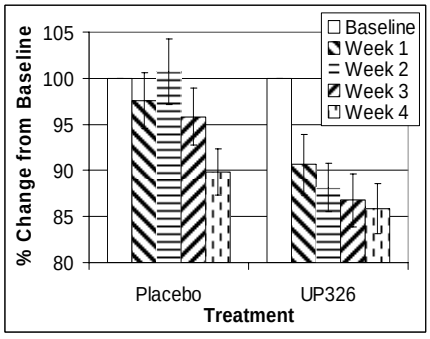


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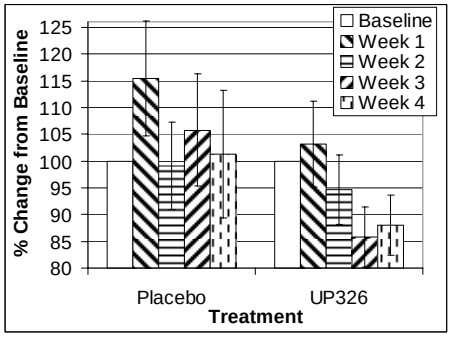
Figure 4



Discussion

Animal and human clinical results suggest that there was significant attenuation of memory deficits and increases in speed and accuracy of processing respectively. These results might be due to a reduction of inflammation and ROS as a result of administration of UP326. Full chronic and acute toxicity testing in animals showed UP326 to have an excellent safety profile (data not shown). The extract is also non-mutagenic by AMES and does not significantly inhibit the CYP450 isoenzymes (data not shown). Studies of brain tissue from aged rats is ongoing in an attempt to correlate the *in vitro* and *in vivo* data.

References

1. Brookmeyer R, Gray S, and Kawas C (1998) *Am J Public Health*, **88(9):** 1337-42.
2. Hebert LE, Beckett LA, Scherr PA, and Evans DA (2001) *Alzheimer Dis Assoc Disord*, **15(4):** 169-73.
3. Ho L, Purohit D, Haroutunian V, Luterman JD, Willis F, Naslund J, Buxbaum JD, Mohs RC, Aisen PS, Pasinetti GM (2001) *Arch Neurol*, **58(3):** 487-92.
4. Hull M, Lieb K, and Fiebich BL (2000) *Expert Opin Investig Drugs*, **9(4):** 671-83.
5. Xiang Z, Ho L, Yemul S, Zhao Z, Qing W, Pompl P, Kelley K, Dang A, Qing W, Teplow D, and Pasinetti GM (2002) *Gene Expr*, **10(5-6):** 271-8.
6. Kalaria RN, Harshbarger-Kelly M, Cohen DL, and Premkumar DR (1996) *Neurobiol Aging*, **17(5):** 687-93.
7. McGeer EG and McGeer PL (1998) *Exp Gerontol*, **33(5):** 371-8.
8. Barnes CA, Markowska AL, Ingram DK, Kametani H, Spangler EL, Lemken VJ, and Olton DS (1990) *Neurobiol Aging*, **(5):** 499-506.
9. McGahon B, Clements MP, Lynch MA **(**1997) *Neuroscience*, **81(1):** 9-16.
10. Murray CA and Lynch MA (1998) *J Biol Chem*, **273(20):** 12161-8.
11. Kulmacz RJ and Lands WE (1983) *Prostaglandins*, **25(4):** 531-40.
12. Needleman P, Turk J, Jakschik BA, Morrison AR, and Lefkowith JB (1986) *Annu Rev Biochem*, **55:** 69-102.
13. Rouzer CA and Samuelsson B (1986) *FEBS Lett*, **204(2):** 293-6.
14. Laufer S, Tries S, Augustin J, and Dannhardt G (1994) *Arzneim-Forsch/Drug Res*, **44:** 629-636.