Exploring the Role of APOE4 Genotype in Chemo Brain or Chemotherapy-Induced Cognitive Impairment

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Abstract

Chemo brain, also known as chemo fog or chemotherapy-induced cognitive impairment, refers to the neuropsychological decline that about 75% patients experience during cancer treatment. It has become an increased area of research interest, because as more patients survive cancer, it is increasingly evident that available treatment methods affect their cognitive function, and subsequently their quality of life. While the biological mechanisms for this phenomenon are still under investigation, there is increasing evidence that the apolipoprotein genotype, specifically the APOE ε4 allele, may be a potential risk factor. APOE ε4 has been identified as the major genetic risk factor in the pathogenesis of Alzheimer's disease, which is also characterized by decline in memory and other cognitive function. Recent research suggests that the APOE ε4 allele, when exposed to chemotherapeutic agents, can impair cognitive behavior and alter brain structure. Particularly, impairment in spatial memory and reduction of hippocampal volume was observed. This review examines and critically analyzes pre-clinical and clinical studies that test the theory that the APOE ε4 allele increases susceptibility to chemotherapy-induced cognitive impairment. Better understanding the role of APOE ε4 in cognitive decline will allow for further research to be conducted on different chemotherapeutic agents as well as the development of preventive measures for APOE ε4 carriers.

Introduction

Chemotherapy-induced cognitive impairment (CICI), also termed "chemobrain" or "chemo fog" by cancer patients, describes the cognitive dysfunction that results from chemotherapeutic agents often used to treat cancer (1). As cancer-related survival rates increase, the prevalence of CICI has increased as well (2), with up to 75% of cancer patients experiencing CICI during treatment, and 35% still reporting symptoms post treatment (3). Some of the symptoms that are commonly observed include a deficit in cognitive domains related to visual-spatial ability, processing and motor speed, memory, concentration and executive function (1). Subsequently, changes are also seen in the brain regions that modulate these cognitive skills, such as the prefrontal cortex, frontal and temporal lobes, and the hippocampal and cortical regions (1, 4).

Assessing and diagnosing CICI has proven to be challenging due to the sometimes transient nature of the symptoms, as well as the extensive number of ways in which cognitive function can be measured (3). While there are no official guidelines to clinically assess CICI (1), the International Cognition and Cancer Task Force (ICCTF) recommends a neuropsychological assessment battery consisting of the following measures: the Trail making Test (TMT) to assess the psychomotor speed and executive function, the Hopkins Verbal Learning Test-Revised (HVLT-R) to assess verbal memory and delayed recall, and the Controlled Oral Word Association (COWA) Test to assess speeded lexical fluency and executive function (3, 5, 6). Other supplemental measures include the Wechsler Adult Intelligence Scale (WAIS) and the Paced Auditory Serial Addition Test (PASAT) (5, 6).

The etiology of CICI is still under investigation, but several characteristics including demographic variables, comorbidities and biological factors, all play a role in the pathophysiology of the disease (1, 3, 5). Age is a known risk factor for cognitive decline, which can also be exacerbated by premorbid psychological issues such as anxiety and depression. Inflammation, altered blood-brain barrier, and cytokine levels have also been associated with CICI (1, 5). Studies investigating the apolipoprotein E (APOE) and catechol-o-methyltransferase (COMT) genes showed a correlation between these genes and a higher susceptibility to cognitive deficit, especially in patients who received chemotherapy treatment (3, 5). The APOE ε4 allele in particular has been linked to several diseases characterized by cognitive dysfunction, as well as poor cognitive outcomes in other neurological conditions like stroke (3).

APOE ε4 genotype

APOE is a glycoprotein that regulates the metabolism of cholesterol and fatty acids and is made up of 299 amino acids (7). APOE levels are typically highest in the liver and the brain, where it is synthesized by astrocytes and sometimes neurons (7). The APOE gene consists of different polymorphisms with three main alleles — APOE ε2, APOE ε3, and APOE ε4 (7) — which can allow for up to six genotype combinations (8). Research has shown the APOE ε4 genotype to be a risk factor for the development of Alzheimer's (1, 7, 8), traumatic brain injury (3, 8) and cerebral amyloid angiopathy (CAA) (7). Other studies conclude that APOE ε4 has neurotoxic effects that can manifest as behavioral deficits in animals (9). The exact mechanism of the APOE ε4 allele is still under investigation, but researchers hypothesize that the ε4 allele lacks in its ability to conduct neuronal repair compared to the other alleles (10). Several human and mouse studies have been conducted to understand the role APOE ε4 plays in CICI, but further research is needed to establish the genotype as a definite risk factor for CICI.

Methods

A literature review was performed using Google Scholar, PubMed and ClinicalKey. Search terms included *chemobrain, chemofog, chemotherapy-related cognitive impairment,*

chemotherapy-induced cognitive impairment, APOE, apolipoprotein E, CRCI and *CICI.* Articles were selected based on impact factor, investigation relevant to CICI and APOE, and a publication date within the last 20 years.

Discussion

APOE ε4 in clinical studies

Over the years, the acute and long-term cognitive changes reported by cancer survivors treated with chemotherapy were attributed to fatigue and other psychological factors (10). However, after controlling for the aforementioned factors, recent literature presents evidence suggesting that these changes are in fact linked to chemotherapy treatment (10, 11, 12, 13). These studies also show that other risk factors like age, pretreatment cognitive reserve and the APOE ε4 allele play a role in the extent of cognitive decline (12, 11, 14). The longitudinal studies conducted assessed visual memory, spatial ability and psychomotor functioning (8), verbal fluency and memory (13), learning and memory domain and cognitive function (12), as well as processing speed and working memory (11, 14).

While examining the detrimental effects of chemotherapy on cognitive function of cancer survivors, Ahles et al. concluded that APOE ε4 carriers are more likely to experience more severe levels of change in cognition for longer periods of time (8, 10). After assessing different neuropsychological domains, individuals with the APOE ε4 allele performed significantly worse than those lacking the ε4 allele in the visual memory and spatial ability domains, with p values of $p<0.03$ and $p<0.05$ respectively (8). There was also a substantial difference in psychomotor functioning across the two APOE statuses, although not statistically significant (8). Findings by Mandelblatt et al. also demonstrated a larger decline in the memory domain of APOE ε4 carriers versus other treatment groups (12). It also took twice as long for APOE ε4 carriers to show improvements in these domains compared to other genotypes (12). In the attention, processing and executive function domain, the interaction of age and APOE status on chemotherapy was evident as the survivors with lowest scores in this domain were older survivors with the APOE ε4 allele (12).

Of the limited number of clinical studies examining the effects of APOE ε4, most show its association to cognitive decline in the context of age and specific chemotherapeutic regimens, but the current evidence is not sufficient to establish a direct correlation between the APOE ε4 allele and worse CICI outcomes. Other chemotherapeutic agents need to be studied individually as well as in conjunction with other agents or as part of common regimens. Additionally, patient reports and neuropsychological batteries provide information of cognitive effects but are limited in the inability to suggest actual biological processes and mechanisms of action. Thus, animal studies are necessary to analyze these mechanisms and structural changes associated with APOE ε4 in CICI.

Effect of APOE ε4 in mouse models

Human APOE knock-in mouse model and CICI

Data supporting APOE as a risk factor for CICI and the lack of reports establishing that chemotherapy-related cognitive decline is directly due to chemotherapeutic agents administered to cancer patients, led researchers to develop a human APOE knock-in mouse model to understand and define the mechanism of APOE in CICI (4). Previous APOE knock-in mouse models have shown that APOE ε4 mice exhibit higher levels of inflammatory cytokines than APOE ε3 mice, as well as deficits in dendritic spine density (4, 15). Biochemical differences in APOE ε4 and APOE ε3 mice are evident, and researchers suggest that this may be due to differences in post-translational modifications (15). However, Speidell et al. sought to explain the how these genotypes interact in the presence of cancer, as well as during the administration of a therapeutic agent.

Thirty-nine human knock-in C57BL/6J mice with APOE ε4 (n=24) and APOE ε3 (n=18) were selected and maintained according to the ethical and national/international guidelines for animal welfare (4). The experimental group of mice was treated with doxorubicin, a common chemotherapeutic agent used for breast cancer. The control group remained untreated. Both groups were assessed with the use of behavioral assays and their brains analyzed via magnetic resonance imaging (MRI).

After the behavioral assay, the mice were euthanized and their brains extracted to conduct an MRI study. This study was completed on 12 control animals with an even APOE ε3 and APOE ε4 distribution as well as 5 APOE ε3 and 6 APOE ε4 treated mice (4). Voxel-based morphometry (VBM) was then used to evaluate the discrepancies in brain anatomy between the treated group versus the untreated group. These anatomical changes were assessed with a T2-weighted sequence. Data acquired via the MRI study enabled researchers to analyze difference in grey matter maps across both groups (4).

When both genotypes were compared with respect to distance traveled in the open field, rearing events, and amount of time spent in the open arm of the maze, there was no substantial difference between the control APOE ε4 and APOE ε3 mice (4). This suggests that inherently, there were no major differences in locomotion, exploration, and anxiety between mice with APOE ε4 versus APOE ε3. These three parameters were also tested at baseline in mice that were treated with doxorubicin a week prior to doxorubicin administration. A decline was evident 1 week post-treatment as the distance traveled and number of rearing events in the open field dropped to almost half that of baseline, in both treatment groups (4). This demonstrates the negative effect of a single dose of doxorubicin on exploration. After the second dose of doxorubicin at the 6-week mark, APOE ε3 show improved exploratory levels, almost similar to baseline. APOE ε4 mice on the other hand, exhibited even worse levels compared to baseline (4). No substantial differences were observed in anxiety levels when compared across both genotypes.

The most notable differences between APOE ε4 and APOE ε3 mice were found in the cognitive behavior trials. Spatial learning and memory were assessed in the treatment group 5 weeks after doxorubicin administration, and APOE ε4 mice showed a much longer latency period than APOE ε3 mice (4). Although the APOE ε4 latency period decreased as the training days progressed, there was still an almost 10-fold

difference in the latency period (100 secs) compared to APOE ε3 (13 secs) with a p value of <0.001 (4). This variation was also evident in the 72-hour probe trial, suggesting that the poor performance noted during the training days affected their ability to learn and retain information on how to locate the target hole. It is evident that doxorubicin treated APOE ε4 mice showed the longest latency periods to locate the escape hole of all groups; however, because the control APOE ε4 mice also showed deficits in spatial learning abilities compared to control APOE ε3 mice, this suggests that the APOE ε4 genotype is a risk factor for spatial learning and memory.

MRI results showed smaller grey matter (GM) volumes in control APOE ε4 mice compared to control APOE ε3 mice. The difference in GM volumes was visible especially on the hippocampus and certain regions of the striatum (4). In the doxorubicin treatment group, imaging showed decreased GM in even more regions of the brain, including the lateral geniculate nucleus and the piriform cortex (4). VBM analyses of the MRI studies comparing the untreated versus the doxorubicin-treated brains revealed that even in APOE ε3 mice there were substantial differences and alterations in GM within the frontal cortex and hippocampus (4). These patterns were also visible in the APOE ε4 mice, but on a larger scale (4).

These data indicate that although both the APOE ε4 and APOE ε3 mice showed learning impairment post-treatment, APOE ε4 mice had a significantly higher magnitude of impairment. Additionally, structural changes such as reduced GM in areas like the hippocampus were evident in both the doxorubicin treated APOE ε4 and APOE ε3 mice but more so in the APOE ε4 mice. This model, if further studied, can be replicated to take into account other factors that could affect the mechanism of APOE ε4 on CICI, including but not limited to age, administration of chemotherapy regimens as seen in clinical practice, and eventually longitudinal clinical trials in cancer patients.

Importantly, this study did not take into account the implications of the administration of doxorubicin in clinical settings, which may be administered at varying doses over a long period of time or concurrently with other therapies. This is important because other factors have been shown to exacerbate CICI, such as hormone therapy (1, 4), or potentially improve cognitive function, like smoking (11). Additionally, non-oncogenic mice were used as the model in this study, and while it was successful in establishing an association between APOE ε4 and CICI, replicating the study in oncogenic mice would be more representative of the interactions and mechanisms between cancer and APOE ε4. Finally, the data in the report were presented using the standard error of the mean (SEM), which does not necessarily depict the variability of the data points and does not accurately establish statistical significance. Using standard deviation (SD) would be more appropriate as a descriptive statistic, as it is more accurate in showing the variability of the sample (16).

Human APOE knock-in mouse model and CICI in the context of aging

As stated above, it would be important to use the APOE knock-in mouse model to study APOE ε4 together with aging as risk factors for CICI. The study was in fact replicated by the same group of researchers to account for age in the breast cancer survivor population by using aged mice and assessing other cognitive areas. The C57BL/6J human APOE knock-in mouse model was used with a bigger sample size of 61 that included 30 APOE ε3 and 31 APOE ε4 mice (17). The two APOE ε4 and APOE ε3 control groups received saline, and the APOE ε4 and APOE ε3 treatment groups received doxorubicin (17).

Some of the behavioral assessments used for this experiment were similar to those performed previously, but the set-up of the assays differed. These tests included the open field test, elevated zero maze, and the Barnes maze. Also, two supplemental assays included pre-pulse inhibition (PPI) and fear conditioning.

After about 31 to 35 weeks post-treatment, the mice were euthanized so that tissue and blood samples could be collected from healthy mice. The brains that were perfused with phosphate buffered saline (PBS) were then prepared and processed for immunohistochemistry and staining with antibodies (17). Those perfused with formalin were used for the MRI study, which was performed and assessed with a T2-weighted sequence. VBM was performed as well to assess GM and white matter (17).

Results from this experiment indicated weight loss in the doxorubicin treatment groups but showed no substantial difference across genotypes (17). At baseline, APOE ε3 mice spent 50% more time at the open zones of the open field and elevated zero maze than APOE ε4 mice, pointing to less levels of anxiety in the APOE ε3 groups compared to APOE ε4. Two weeks after treatment, both doxorubicin-treated and saline control groups traveled the same distance in both assessments that evaluated anxiety and exploration (17). Fifteen weeks post-treatment, all groups showed similar distance travelled in both apparatuses as well. Also, all groups showed a reduction in the amount of time spent in the exposed zones of the elevated zero maze post treatment (17); therefore, according to this study, the chemotherapeutic agent has minimal effect on locomotion and anxiety.

The PPI data showed that there was no difference in the degree of PPI between both genotypes at a baseline of 3 decibels. (17). The doxorubicin group did not experience any significant differences in PPI compared to the saline control group (17). Both genotypes in the treated and untreated group initially explored the Barnes maze at very similar paces (17). However, the treated APOE ε3 was much slower than the untreated APOE ε4 group ($p = 0.004$) (17). Also, the control APOE ε3 mice were faster at escaping the hole than the control APOE ε4 mice, especially on training days (TD) 1 & 2 (17). This shows that without any chemotherapeutic agents, spatial learning is better in APOE ε3 mice, but an untreated APOE ε4 mouse will perform better than a doxorubicin-treated APOE ε3 mouse. Doxorubicin treatment showed no diminishing effect on the latency of APOE ε3 mice throughout all four TDs, meanwhile significant latency was observed in treated APOE ε4 mice (17). Treated APOE ε4 mice also had a harder time escaping the hole in the Barnes maze without some guidance (17). This suggests that the treatment impairs the spatial learning ability of mice with the APOE ε4 genotype but does not affect those with APOE ε3. The results of the probe trial conducted 72 hours after the Barnes maze tests indicated that doxorubicin treatment in both genotypes correlated with increased latency in the ability of the mice

to enter the target hole (17). Some of the treated mice failed to complete this task at all, alluding to poor retention and deficiency in spatial memory.

VBM analysis was performed from MRI data, and there was no evidence of significant morphological alterations in brain areas of doxorubicin-treated APOE ε3 mice. The APOE ε4 mice, however, showed some GM atrophy in the frontal cortex, when compared to the control group (17). Although these findings were not statistically significant, when all four experimental groups were compared, APOE ε4 had lower GM volumes in the cerebral cortex and striatum, but no differences in the hippocampus.

This replication study supported some of the conclusions made in the previous study, but also provides an alternate view for others. Both reports demonstrated decreased performance on spatial learning in APOE ε4 mice that received chemotherapy, which aligns with clinical symptoms that breast cancer patients have reported during and post chemotherapeutic treatment. This study was successful in using a new preclinical model to present data on the intersection of age and APOE ε4 in the pathogenesis of CICI. It also revealed that the structural changes seen in patients with CICI or in mouse models may be transient and not long lasting. While this seems promising, more research is necessary to consider other variables like premorbid conditions and regimens with multiple chemotherapeutic agents. The methodology of neither study allowed for an extensive look at the effects of the chemotherapeutic at every single point from the moment treatment was administered to the end of the 35 weeks for which assessments were conducted. Furthermore, both studies focused on homozygous genotypes for APOE ε4 and APOE ε3 only and did not account for other polymorphisms and their possible effect on cognitive function.

Conclusion

The development of better chemotherapeutic agents and cancer treatment regimens has resulted in improved outcomes for cancer patients. Research shows that while some of these treatments allow patients to live longer, they can also have debilitating side effects that greatly impact quality of life. Breast cancer chemotherapeutic agents, in particular, have been shown to cause cognitive dysfunction in patients during and after treatment. The etiology of this chemotherapy-induced cognitive impairment has not yet been established, but there are several associated mechanisms that have been suggested. Some studies indicate that neuronal injury and a lack of plasticity (10), elevated levels of inflammation and circulating cytokines (19), and genetic factors such as APOE ε4 and COMT (3, 5) may play a role.

APOE ε4 has been implicated both in preclinical studies and human clinical trials as a potential risk factor for cognitive decline in general, especially in relation to the cognitive changes seen with aging and Alzheimer's disease. Research has also shown that APOE ε4 is associated with increased susceptibility of CICI for long-term cancer survivors (8, 11, 20). Studies by Speidell et al. using the APOE knock-in mouse model suggests that the APOE ε4 genotype predisposes patients to cognitive dysfunction, especially in response to chemotherapeutics such as doxorubicin (4, 17, 21, 22). These data show that doxorubicin-treated APOE ε4 mice exhibited significant impaired spatial learning compared to the other experimental groups, as well as poor memory retention (4). Changes in brain structure were also evident as seen in GM volume in the hippocampal regions and the striatum (4). There was compelling evidence suggesting that the APOE ε4 on its own shows decreased cognitive function, which is exacerbated when chemotherapy is administered.

The study by Demby et al. supplements the findings by Speidell using the APOE knock-in mouse model. The APOE ε4 also showed higher levels of cognitive impairment in relation to the APOE ε3 genotype. This deficit in spatial learning worsened with doxorubicin treatment. However, one major discrepancy between the studies is the lack of structural changes in the brain observed in the second study. The researchers attribute this to the fact that mice were studied over a long period of time, and there is a possibility that the cerebral alterations seen are just short term (17). Also, the former experiment noted significant GM reductions in the hippocampus region, which was more pronounced in APOE ε4 mice (4). However, the researchers who replicated the study with the APOE knock-in mouse model reported no findings that showed differences in hippocampal volumes between both groups. The discrepancy could be attributed to the fact that the one experiment was conducted over a long period of time or to a slight difference in the behavioral assays. In summary, development of this APOE knock-in C57BL/6J mouse model and the data that has resulted from its use will pave the way for further research. Future studies that are conducted over a longer period of time, that account for premorbid conditions, and that utilize oncogenic mice will potentially yield supplemental information that can be used to consider establishing APOE ε4 as a risk factor for chemo brain.

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