Cannabis use is associated with increased CCL11 plasma levels in young healthy volunteers

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A R T I C L E   I N F O

Article history:
Received 27 April 2013
Accepted in revised form 17 June 2013
Accepted 18 June 2013
Available online 29 June 2013

Keywords:
Aging
Cannabis
CCL11
Chemokine
Psychosis
Schizophrenia

A B S T R A C T

Cannabis is a widely used recreational drug. Its effect on human health and psychosis remains controversial. In this study, we aimed to explore the possibility that cannabis use influenced CCL11 plasma levels. Increased CCL11 chemokine has been reported in schizophrenia and cannabis is a known trigger of schizophrenia. Additionally, plasma levels of the chemokine CCL11 have recently been shown to increase with age and with cognitive deficits and hippocampal neurogenesis. For this study, a total of 87 healthy volunteers (68% men, age range 18–35 years) completed the Cannabis Experience Questionnaire that included information on sociodemographic and morphometric data and provided a blood sample for CCL11 measurement. ‘Current users’ of cannabis (n = 18) had significantly higher CCL11 plasma levels compared to ‘past users’ (n = 33) and ‘never users’ (n = 36) [F(3,84) = 3.649; p = 0.030]. The latter two groups had similar CCL11 levels. Higher CCL11 plasma levels could not be attributed to gender, age, body mass index, physical activity or use of other legal/illegal drugs. These results suggest that cannabis use increases CCL11 plasma levels and the effects are reversible when cannabis use ceases.

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1. Introduction

Cannabis is a widely used illegal drug and at the heart of an intense debate about the legalization of its use. Deleterious effects on mental health have been extensively reported, such as schizophrenia onset (Moore et al., 2007); however, its therapeutic benefits in some conditions are also recognised (Borgelt et al., 2013). A better understanding of the biological effects of chronic cannabis could therefore be useful.

Cannabis use has been linked to structural brain changes, including a significant dose-dependent reduction in size of the hippocampus and amygdala (Batalla et al., 2013). In animal models, hippocampal decrease in neural volume, neuronal and synaptic density and dendritic length of CA3 pyramidal neurons (Landfield et al., 1988) has also been described with cannabis administration. A similar pattern has also been seen in human normal aging (Hof and Morrison, 2004). The mechanisms by which cannabis might cause structural brain changes remain unclear.

We were interested in evaluating cannabis-triggered biomarkers that might correlate with brain changes seen in mental disorders. The chemokine CCL11, also known as eotaxin-1, is secreted by immune cells and is involved in allergic reactions. Its plasma levels are influenced by several factors, including glucose metabolism and insulin plasma levels (Choi et al., 2007), fat deposition (Kato et al., 2011) and physical activity (Ghanim et al., 2010). Increased CCL11 has also been described in schizophrenia (Teixeira et al., 2008) compared to other chemokines, such as CCL2 or CCL3, although the cause remains unclear. More recently, CCL11 has also been described to play a key role in adult neurogenesis and cognitive functions (Villeda et al., 2011), along with other chemokines such as CCL2, CCL12, CCL19, haptoglobin and b-macroglobulin. Animal studies have shown CCL11 plasma levels were inversely correlated to hippocampal neurogenesis and learning and memory. It has also been established that CCL11 also increases with age in both humans (Shurin et al., 2007; Villeda et al., 2011) and rodents (Jeon et al., 2012). Interestingly, these studies...
also suggested that plasma levels of CCL11 might serve as a peripheral biomarker for neurogenesis. There is a growing interest on the CCL11 chemokine, as it has been described increased in schizophrenia and also in aging process and inversely correlated to hippocampal neurogenesis and learning. Smaller hippocampus compared to healthy volunteers has been widely described in schizophrenia and also in cannabis users (Batalla et al., 2013).

In this study, we explored the impact of present and past cannabis use on CCL11 plasma levels. We hypothesized that cannabis use may increase CCL11. In a group of young healthy volunteers, we analysed CCL11 plasma levels and the use of cannabis, taking also into account potential confounding factors such as smoking or the use of other illegal drugs.

According to the use of cannabis, the total sample was divided in three groups for the analyses: subjects with no contact to cannabis (‘never use’ group), subjects using cannabis at present (‘current use’ group) and subjects who had not consumed cannabis for 2 months or more (‘past use’ group). All ‘current user’ subjects had used cannabis in the last 30 days. None had cannabis between 1 and 2 months.

2. Materials and methods

2.1. Sample

This study was carried out as part of the Biomarkers in Early Psychosis study. Healthy volunteers were recruited from November 2008 to July 2012 via advertisements posted in the city of Cambridge, UK. The Local Research Ethics Committee approved the study and in accordance to the code of ethics of the World Medical Association (Declaration of Helsinki).

Exclusion criteria included: (1) prior personal or family history of psychotic disorder, (2) being prescribed with psychotropic medication (any), (3) history of head injury, (4) history of major medical problem and (5) learning disability leading to specialist care. Subjects were contacted by phone screened for eligibility criteria before the interview and again during the study assessment.

All participants provided written informed consent prior to the study. All volunteers participated in a 1-h testing at the Addenbrooke’s Centre for Clinical Investigation in Cambridge, UK.

2.2. Clinical assessments

Participants underwent physical and physiological measurements (e.g. weight, height, waist circumference, heart rate and blood pressure), followed by a venepuncture for metabolic syndrome markers and a series of questionnaires. Below are those relevant to this study.

Cannabis Experience Questionnaire (CEQ) Barkus et al. (2006). Participants completed a validated questionnaire that assessed current and past use patterns of cannabis and other illegal drugs. These included a detailed history and frequency of use of cannabis and other illegal drugs, type of cannabis consumed and experiences produced on each drug.

General Practice Physical Activity Questionnaire (GPPAQ) (2006). This is a validated screening tool for use in primary care that can be used to assess adult (16–74 years) physical activity levels. It generates a 4-level Physical Activity Index (PAI): active (4), moderately active (3), moderately inactive (2) and Inactive (1).

Current and past use of tobacco and alcohol was also recorded, including length of use and amount.

Calgary Depression Scale (CDS). The CDS is a 9-item scale developed for assessment of depression in schizophrenia (Addington et al., 1990). A cutoff score of 6 points has been proposed for a diagnosis of clinical depression. None of the volunteers met the criteria for clinical depression.

2.3. Laboratory-based measures: CCL11 measurement

Lithium heparin blood samples were centrifuged for 15 min, and plasma was transferred to a secondary tube. The plasma was stored at –80 °C until analysis. None of the samples underwent more than two freeze–thaw cycles prior to acquisition of CCL11 levels. Biochemical markers of metabolic syndrome (random glucose level, total cholesterol, etc.) were also analysed.

2.4. CCL11 Assay

CCL11 plasma concentrations were determined using an ELISA assay by the NIHR Cambridge BRC Core Biochemical Assay Laboratory. The assay was a commercial sandwich ELISA kit (CCL11 Quantikine kit, R&D Systems, Minneapolis, USA). A monoclonal antibody specific for CCL11 was pre-coated onto a microplate, and 100 μl of assay diluent and 50 μl sample were pipetted into the wells in duplicate. Samples were incubated for 2 h at room temperature; during this time, CCL11 present in the samples was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for CCL11 was added to the wells and incubated for 1 h at room temperature. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of CCL11 bound in the initial step. The colour development was stopped, and the intensity of the colour was measured on a Victor 3 plate reader (Perkin Elmer). CCL11 results were calculated using the Multicalc software package (Perkin Elmer). All experiments were performed under blind and randomised conditions. Two human plasma pools were used as quality control samples. The between-assay coefficients of variation were 4.9% at a concentration of 84 pg/mL and 5.9% at 211 pg/mL (n = 10).

2.5. Statistical analysis

Descriptive results are presented as mean and standard deviation (SD) or percentages. Variables were assessed for normality. Significance was defined as p < 0.05 for all statistical tests, and these were performed using version 18.0 of SPSS (Statistical Package for Social Sciences) for Mac.

Groups (‘never use,’ ‘past use’ and ‘current use,’ see above) were compared using the non-paired Student’s t-test or a one-way ANOVA as appropriate, the χ² test for comparisons of proportions, or Pearson’s r for correlations, as described in the results sections. In the ANOVA test, post hoc analysis and linear tendency (polynomial) tests were also performed. Multiple regression analyses are described in the Results section. Cannabis is frequently smoked blended with tobacco, so we explored ways to differentiate between the impacts of the two factors (tobacco and cannabis) on CCL11 plasma levels. As cocaine has been associated with accelerated brain aging, we explored this particular drug independently, as described in results.

3. Results

3.1. Demographics

The final study cohort comprised 87 healthy subjects (59 men, 68%) with a mean age of 23.6 years (SD) = 4.6, range 18–35. Table 1 shows the morphometric, physical activity index and main results of these subjects. In the whole sample (n = 87), CCL11 plasma levels were not associated with age [r(85) = 0.060, p = 0.580], degree of physical activity [r(85) = 0.177, p = 0.121], BMI [r(85) = −0.196, p = 0.130], gender [t(2,85) = 1.595, p = 0.114] or random glucose plasma levels [r(85) = −0.053, p = 0.634]. The correlation between same variables (age, physical activity, BMI, gender and random glucose plasma levels) and CCL11 plasma levels in each of the groups showed no
statistical significance either, except for the CCL11 and age in the past users group ($r^2(31) = 0.346$, $p = 0.048$).

The sample was divided among those who have never taken cannabis ($n = 36$, 68% men), only in the past ($n = 33$, 66% men) and current cannabis users ($n = 18$, 66% men). By one-way ANOVA test, no differences were found among the three groups with regard to physical activity index ($F(3,84) = 1.685; p = 0.192$), BMI ($F(3,84) = 0.726; p = 0.487$) or age ($F(3,84) = 1.139; p = 0.325$). Groups that differed in tobacco use (see Table 1) were thus considered as a confounding factor, thereafter.

3.2. Cannabis use and level of CCL11

One-way ANOVA indicated differences between the three groups ($F(3,84) = 3.649; p = 0.030$). Table 1 shows mean CCL11 plasma levels in the three groups. Differences followed a significant linear association between cannabis exposure and CCL11: mean CCL11 level increased with the degree of exposure (polynomial linearity test, $p = 0.009$). Least significant difference (LSD) post hoc analyses were significant for those currently using cannabis compared with both past users ($p = 0.038$) and to never users group of cannabis ($p = 0.019$). Past-use and never-use groups did not differ ($p = 0.534$). We also compared the ‘current use’ group ($n = 18$) with the non-current users ($n = 69$, merging past and never use groups): current user had greater CCL11 plasma levels [138.44 pg/mL (SD = 60.48) vs. 110.48 pg/mL (SD = 33.03); $t = 2.638$; $p = 0.01$]. In the ‘past use’ group ($n = 33$), CCL11 plasma levels were not correlated to the length of time without using cannabis [CEQ Q7; $r(31) = -0.084; p = 0.648$].

3.3. CCL11 plasma levels and use of tobacco

Table 1 shows that the three groups differed in numbers of cigarettes. In our sample, just one subject smoked tobacco and had never used cannabis, so a direct comparison between two groups (smokers of only tobacco vs. other groups with cannabis use) was not possible. There was no statistically significant correlation between current cigarettes per day with CCL11 plasma levels [$n = 87$, $r(85) = 0.155$, $p = 0.158$]. We performed the same correlation with a restricted group that included only subjects who were former cannabis users but still smoking tobacco; the current number of tobacco cigarettes smoked per day was not correlated to CCL11 plasma levels [$n = 33$, $r(31) = -0.170; p = 0.336$].

We used a multiple regression analysis to confirm that the association between cannabis use and CCL11 was not confounded by tobacco use. In a model with CCL11 as the dependent variable and including age, gender, current tobacco cigarette consumption and cannabis use (never, past use, present use), only cannabis was significantly associated with CCL11 ($p = 0.047$); tobacco use was not ($p = 0.6$). Similar results were observed with a regression model including current use of cannabis versus never and past use as a single category: current use was significant $p = 0.038$, whereas number cigarettes ($p = 0.5$), gender ($p = 0.13$) and age ($p = 0.4$) were not. We also explored the effect of fat deposition in CCL11 plasma levels, using CCL11 as dependent variable and age, gender, category of cannabis use and BMI as independent variables. Number of cigarettes was not included as it correlated highly with cannabis use in our sample. Only category of cannabis use was significant. A similar model using waist circumference rather than BMI showed the same results.

3.4. CCL11 plasma levels and other drugs

In order to explore the impact of the use of other illegal drugs, we focused on individuals not currently using cannabis (never or past use, $n = 65$). Of those, 48 had never taken any other drug whereas 17 reported past history of other drug use. There was no statistical difference between the two groups using a $t$-test [$108.83 pg/mL SD = 34.00 vs. 117.29 pg/mL SD = 32.29, t(2,63) = -0.811; p = 0.4$). Among the subjects not currently using cannabis ($n = 65$), 10 reported current use of cocaine defined as at least one use during the last year. CCL11 levels among cocaine users were higher compared with non-users but did not reach statistical significance [$121.70 pg/mL SD = 34.78 vs. 109.25 pg/mL SD = 32.74, t(2,63) = -0.999; p = 0.375$]. Among the 18 people currently using cannabis, those who had never used cocaine ($n = 5$) had similar CCL11 plasma levels (144.04 pg/mL SD 73.24) compared with those who had used cocaine ($n = 13$); $p = 0.011$. Only one subject was currently using cocaine defined as use within the past week. Similar results for CCL11 plasma levels were obtained for amphetamine users (results not shown).

4. Discussion

In this study, cannabis use in healthy volunteers was associated with CCL11 plasma levels. The differences between groups did not appear to be attributed to known confounding factors such as the use of tobacco, use of other illegal drugs, BMI, age, gender or physical activity.

Due to our cross-sectional study design, the fall in CCL11 levels in the ‘past use’ group compared with the ‘current use’ group deserves further discussion. In our view, the most likely explanation is that the cannabis effect on CCL11 levels fades away over time; we could not find a direct correlation between the length of time without using cannabis and CCL11 plasma levels, even after correcting for the degree of use (data not shown). This plausible explanation is in line with clinical and experimental results: neuropsychological profile improves after stopping use of cannabis (Tait et al., 2011). Nevertheless, a prospective study would be necessary to confirm these results.

Special attention was paid to the effect of tobacco, which is commonly used alongside cannabis. As expected, the use of cannabis and tobacco was correlated in our sample. However, cannabis significantly increased CCL11 whereas tobacco had no significant effect, as it was seen in the group who were ‘current tobacco smokers and past users of cannabis.’ However, cannabis is often consumed along with tobacco so whether there is a potentiation between both compounds remains unclear.
With regard to other illegal drugs, our results suggested that cannabis impact on CCL11 is independent of the use of cocaine and amphetamine. However, the limited sample size of subjects using other drugs or the underreporting of the use of some of them (such as cocaine) might have contributed to lack of significant results and therefore conclusions might be taken cautiously.

We found no correlation with age in our study. CCL11 plasma levels increase with age in humans (Shurin et al., 2007; Villeda et al., 2011) and in mice (Jeon et al., 2012). However, CCL11 differences with age in humans have been observed when there are a few decades of difference between groups (Shurin et al., 2007; Villeda et al., 2011). Here, we chose a narrow age group (from 18 to 35 years old) because we tried to avoid this confounding factor and only to study the effect of cannabis alone on biological aging. Our results suggest a potential link between cannabis use and an aging phenotype through increased CCL11 plasma levels. In our sample, CCL11 plasma levels for the ‘current use’ group resembles those to be expected in individuals about three decades older (Shurin et al., 2007), while CCL11 levels in the ‘never use’ group were similar to what is expected for their age group (20-29 years old).

Another interesting yet speculative approach is considering CCL11 as a factor linked to brain aging. Brain aging has been linked to the use of illegal drugs and with mental disorders. For instance, a recent report linked cocaine use to accelerated brain aging (Ersche et al., 2012) and also cellular aging has also been linked to almost all severe mental disorders, including bipolar disorder (Yatham et al., 2009), major depression (Simon et al., 2006) and, more notably, schizophrenia (Kirkpatrick et al., 2008). In any case, although increased CCL11 has been described in schizophrenia (Teixeira et al., 2008), there are no studies of CCL11 plasma levels in schizophrenia with and without cannabis use.

The way by which CCL11 might be involved in the deleterious effect of cannabis on brain functioning and mental health disorder also needs to be elucidated. CCL11 produced by T helper type 2 (Th2) cells at the choroid plexus has recently been linked to both brain aging and cognitive decline (Baruch et al., 2013). Persistent cannabis use has been consistently associated with neuropsychological decline broadly across domains of functioning (Meier et al., 2012). Memory impairments have been associated with the duration, frequency, dose and age of onset of cannabis use (Solowij and Battist, 2008), indicating a profile of linear worsening associated with greater and accumulative use. We found a similar linear pattern in our sample with regard cannabis use and CCL11 plasma levels.

This study has some limitations. For instance, drug screening was not performed, and therefore all information regarding cannabis use relied on volunteers’ answers. However, we believe it would have increased the number of false-negative (subjects rated as non users when they were actually using cannabis) and therefore resulted on lack of differences. On the other hand, we cannot exclude underreport of other illegal drug such as cocaine. No structured interview was used ruling out comorbid psychopathology. However, none of the subjects were or have ever been under the care of mental health services and clinical assessment by an experienced consultant psychiatrist (EFE) ruled out clinical relevant symptomatology. Mood abnormalities were excluded using Calgary Depression Scale. Acute insulin effects of medical cannabis. Pharmacotherapy 2013;33:195–209.

5. Conclusion

In conclusion, this preliminary study suggests that current cannabis use is linked to increased CCL11 plasma levels. This chemokine is linked to the aging process, and it is easily measurable in human blood. The precise mechanism by which cannabis increases CCL11, its role on neuropsychological deficits and on psychiatric disorders warrants further investigation.

Acknowledgements

We want to thank the Brain Behaviour Research Foundation for their funding through 2009 NARSAD Young Investigator Award.

References


