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Intracerebral Stem Cell Administration Inhibits Relapse-like Alcohol Drinking in Rats

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Abstract

Study describes the blockade of relapse-like alcohol drinking by mesenchymal stem cells (MSCs). High alcohol-intake bred rats consumed alcohol for 3 months and were subjected to repeated alcohol deprivations for 7–14 days, followed by alcohol reaccess. Upon reaccess, animals consumed 2.2 g alcohol/kg in 60 minutes. A single intra-cerebroventricular MSC administration inhibited relapse-like drinking up to 80–85% for 40 days ($P < 0.001$). An alcohol-use-disorder was prevented.

Chronic alcohol administration results in marked increases in brain reactive-oxygen species (ROS) neuroinflammation and neurodegeneration (reviewed by Crews and Vetreno, 2016). These changes are postulated to perpetuate chronic alcohol drinking (Crews *et al.*, 2015). This view is supported by studies showing that the systemic administration of lipopolysaccharide to mice leads to marked brain inflammation (Qin *et al.*, 2007) and increases voluntary alcohol intake (Blednov *et al.*, 2011). Thus, means of reducing ROS and neuroinflammation could conceivable reduce alcohol intake.

In tissues, inflammation and ROS coexist and potentiate each other, since TNF-α uncouples mitochondria leading to an increased generation of ROS. In turn, the oxidation of IκB by ROS activates NF-κB translocation into the nucleus, increasing the synthesis of pro-inflammatory cytokines (Kastl *et al.*, 2014). The administration of *N*-acetyl cysteine, a precursor of cysteine and glutathione, two strong antioxidants (Moriarty *et al.*, 2003), was recently shown to inhibit by 70–75% the chronic 24-hr alcohol intake (Quintanilla *et al.*, 2016). These studies did not determine the effects of *N*-acetyl cysteine on a relapse-like drinking paradigm.

Developments in the stem cell field have shown that most tissues contain MSCs, required to maintain tissue homeostasis (Prockop *et al.*, 2010). MSCs can be isolated and expanded from a number of tissues, such as bone marrow and adipose tissue (Ezquer *et al.*, 2015, 2016). MSCs are known to reduce oxidative stress (Valle-Prieto and Conget, 2010) and to secrete anti-inflammatory cytokines including IL10 (Lee *et al.*, 2016) and a soluble TNFα receptor, which neutralizes TNFα (Yagi *et al.*, 2010).

On above basis, it is hypothesized that MSCs expanded and injected intra cerebro-ventricularly will: (a) survive in the brain of rats consuming alcohol chronically and (b) inhibit the intake of large amounts of alcohol, akin to relapse-like drinking, in the 'alcohol deprivation condition', (c) without affecting fluid homeostasis or weight gain.

METHODS

Female Wistar-derived UChB (University of Chile Bibulous) rats selectively bred for their alcohol preference (Quintanilla *et al.*, 2006) weighing 220–280 g were used. The term alcohol corresponds to ethyl-alcohol or ethanol. Studies were approved by the Ethics Committee of the Faculty of Medicine (Protocol CBA#0507).

Bone marrow-derived and adipose tissue-derived MSCs were prepared and characterized as described earlier (Ezquer *et al.*, 2015, 2016). In initial studies, to determine MSCs intraventricular distribution and viability, the cells were labeled with a green fluorescent label (CFSE carboxyfluorescein succinimidyl ester) prior to injection.

Rats under anesthesia were mounted on a stereotaxic frame and injected with 5 µl containing 5×10^5 MSCs in PBS-10% of rat plasma into the left lateral ventricle (A −0.8; L −1.6; V −3.4 according to Paxinos and Watson, 1998). Control animals received 5 µl of vehicle.

Animals that had been under continuous access to 10% v/v alcohol and water for 73 days followed by 27 days of access to 10% and 20% v/v alcohol and water received a single intracerebroventricular (ICV) injection of vehicle (n = 6 rats, control group), bone marrow-derived MSCs (n = 4 rats) or adipose tissue-derived MSCs (n = 4 rats) into the left lateral ventricle. Animals were allowed to ingest both alcohol solutions and water for 10 additional days. Alcohol consumption was expressed as g alcohol/kg/day. Fluid intake and body weight were determined prior to and after MSC administration.

To determine the effect of MSCs *on the 'alcohol deprivation effect'* relapse-like drinking model, (Spanagel and Hölter, 1999; Rodd-Henricks *et al.*, 2001) a new group of rats under 73 days of free-choice 10% alcohol access followed by 13 days of free-choice 10% and 20% v/v alcohol, were deprived of alcohol for 14 days. On Day 4 of deprivation animals received ICV: adipose tissue-derived MSCs (n = 5), bone marrow-derived MSCs (n = 5) or vehicle (n = 5). At the end of the 14 days of alcohol deprivation (10 days after MSCs injection) alcohol solutions were again offered (re-access #1) and alcohol intake was determined in the first 60 minutes. Animals were subsequently subjected to three additional cycles of 3 days of alcohol drinking and 1 week of deprivation prior to three subsequent reaccess periods.

Data were expressed as means ± SEM. Statistical differences were analyzed by a two-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Bone marrow- and adipose tissue-derived MSCs administered intra cerebro-ventricularly: (a) survive and become attached in rat cerebral ventricles, (b) do not affect animal health and (c) reduce 24-hr alcohol intake

Figure 1A shows the MSCs in the left cerebral ventricle 24 hr after injection. Cell viability was confirmed by the presence of intact nuclei (DAPI 4,6 diamino-2-phenylindole stain) in the MSCs labeled green attached to the choroid plexus (CP). Histological studies conducted 7–10 days after MSCs injection did not evidence fluorescent cells in the cerebral ventricles, but do not discard migration to other sites or fluorescence dye marker loss (not shown). Figure 1B shows that MSCs did not affect total fluid intake ($F_{\text{treatment (2, 154)}} = 0.72$, N.S.) or animal weight gain ($F_{\text{treatment (2, 154)}} = 2.46$, N.S.), while body weight increased along time in all groups ($F_{\text{day (13, 154)}} = 2.18$, $P < 0.01$). Figure 1C shows a significant inhibition exerted by both types of MSCs on alcohol intake ($F_{\text{treatment (2, 156)}} = 95.16$, $P < 0.0001$). No significant difference on alcohol intake between the different MSCs groups was seen ($F_{\text{treatment (1, 78)}} = 2.552$, $P = 0.114$, N.S.). The inhibitory effect of MSCs on chronic alcohol intake was seen 24-hr after MSC administration ($P < 0.01$; Fig. 1C). A single dose of MSCs reduced by 50% alcohol intake for 10 days.

Data indicated that viability of long-term MSC action without the need to extend the above studies, since the 24-hr intake pattern induces blood alcohol levels that do not exceed 50 mg/dl, in control rats (see Quintanilla *et al.*, 2016), thus akin to social drinking. Thus, in a new group it was tested whether MSCs would inhibit the marked increases in alcohol intake, akin to relapse-like drinking following alcohol re-exposure under an 'alcohol deprivation effect'

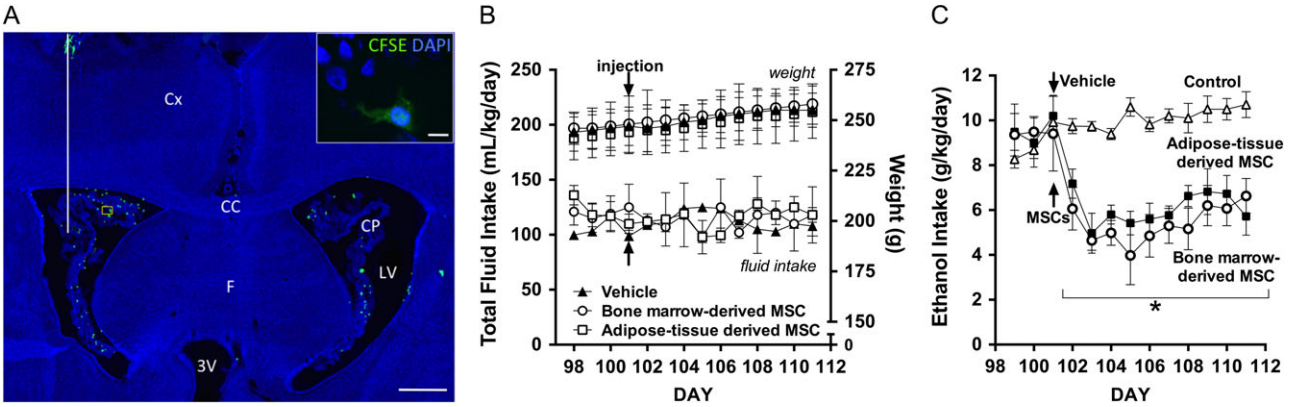

Fig. 1. Intra cerebro-ventricular administration of MSCs to rats consuming alcohol chronically: MSC localization, animal health and pharmacological action. (**A**) Detection of MSCs 24 hr after intraventricular injection. A representative microphotograph of a brain coronal section of an UChB rat after a single injection (5 µl of a solution containing 5×10^5 bone marrow-derived MSCs) into the left lateral ventricle is shown. Bone marrow-derived MSCs were labeled with CFSE (green fluorescence) and DAPI-stained nuclei (blue fluorescence) and visualized in a Nanozoomer- XR slide scanner (Hamamatsu) (scale Bar 500 µm). Inset shows MSCs CFSE/DAPI double labeled, apposed on CP, (scale bar 10 µm). Cx, cortex; CC, corpus callous; CP, Choroid plexus; LV, lateral ventricle; V, third ventricle; F, fornix. White line shows injection site. (**B**) (**Top**) Total fluid intake was not reduced by the administration of bone marrow-derived MSCs or adipose tissue-derived MSCs. Values represent the mean ± SEM of total fluid intake of animals injected with bone marrow-MSC, adipose-MSC or vehicle. Total fluid intake was not affected by MSCs (N.S.). The arrow represents the time of MSC or vehicle administration. (**B**) **(Bottom)** Body weight or weight gain was not reduced by the administration of bone marrow-derived MSCs or adipose tissue-derived MSCs. Values represent the mean ± SEM of body weight (g). Empty circles represent the bone marrow-derived MSC treated group, black squares represent the adipose tissue-derived MSC treated group and empty triangles represent vehicle treated group. Body weight gain was not affected by MSCs (N.S.). (**C**) Intra cerebro-ventricular injection of bone marrow-derived MSCs or adipose tissue-derived MSCs reduces chronic 24-hr alcohol consumption. Rats under chronic alcohol free choice access were injected with bone marrow-derived MSCs, adipose tissue-derived MSCs or vehicle. Data show a reduction of alcohol intake compared to vehicle during all MSCs post-treatment days.

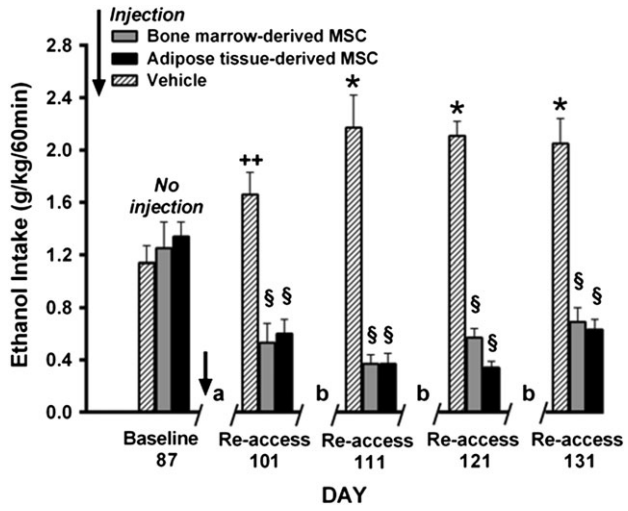

Fig. 2. Intra cerebro-ventricular injection of bone marrow-derived MSCs or adipose-derived MSCs block relapse-like alcohol intake on a 60-minute reaccess. Rats under 87 days of free-choice alcohol access were injected with bone marrow-derived MSCs (gray columns), adipose tissue-derived MSCs (black columns) or vehicle (dashed columns) and deprived of alcohol for 14 days, after which alcohol reaccess was allowed. Animals were further subjected to three additional cycles of 3 days of alcohol drinking and 7 days of deprivation prior to alcohol reaccess. Spacing and letters (a) and (b) in the Figure indicate the number of days of total alcohol deprivation (a): 14-day, (b) 7-day deprivation. $^{*}P < 0.001$ and $^{++}P < 0.05$, indicate alcohol intake increases versus baseline. $^{\S}P < 0.001$, indicates a reduction of alcohol intake compared to baseline value for each of the MSC types, reaching an inhibition of relapse-like alcohol intake up to 80–85%.

condition. The first determination of relapse-like drinking on the alcohol reaccess was conducted 10 days after the injection of MSCs *(vide infra)*.

Bone marrow-derived and adipose tissue-derived MSCs block alcohol relapse-like drinking induced in the alcohol deprivation effect condition

Control rats displayed a basal alcohol intake of 1.14 ± 0.13 g/kg/60 min after 87 days of alcohol free choice access (Fig. 2), intake which was significantly increased after repeated alcohol deprivation and reaccess cycles ($F_{\text{re-access (4, 20)}} = 8.6$, $P < 0.0003$) reaching 2.2 g alcohol/kg/60 minutes. A two-way ANOVA (MSCs treatment × reaccess cycle) of all data in Fig. 2 revealed that animals treated with MSCs significantly reduced up to 80–85% their relapse-like alcohol intake compared to control rats ($F_{\text{treatment (2, 60)}} = 119.4$, $P < 0.0001$). Overall, there were no significant differences between the effects of bone marrow-derived MSCs (gray columns) and adipose tissue-derived MSCs (black columns). Figure 2 also indicated that a single ICV injection of both MSC types significantly inhibited relapse-like drinking for the 40 days investigated, suggesting a protracted remodeling.

DISCUSSION

The administration of MSCs did not unduly influence animal health as shown by the constant fluid intake and a normal animal growth versus controls. The administration of a single dose of bone marrow- or adipose tissue-derived MSCs reduced continuous chronic alcohol intake by 50% during 10 days. A significant inhibition of intake was observed within the first 24-hr of MSCs administration, an effect that mimics a recent report that administration of *N*-acetyl cysteine markedly inhibits chronic alcohol intake within the first 24-hr of receiving *N*-acetyl cysteine (Quintanilla *et al.*, 2016).

Roberts-Wolfe and Kalivas (2015) reviewed studies showing that *N*-acetyl cysteine is effective in reducing the chronic- and cued-induced intake of most drugs of abuse. Such an effect is due to the increases and normalization of the astrocyte glutamate GLT1 transporter, which restores drug-induced alterations in glutamate homeostasis. Low GLT1 levels and high glutamate levels are also observed in rats consuming alcohol chronically (Sari *et al.*, 2013). Whether the protracted effect on alcohol intake that a single MSCs administration exerts, whether via an antioxidant or an anti-inflammatory effect, occurs by normalization of the GLT1 glutamate transporter remains to be determined.

The relapse-like drinking model used in these studies was the 'alcohol deprivation effect' (Spanagel and Hölter, 1999; Rodd-Henricks *et al.*, 2001), a condition in which large amounts of alcohol are consumed in a short period: 2.2 g alcohol/kg/60 minutes in the present study, akin to the relapse-like drinking upon alcohol reaccess in humans. MSCs reduced up to 80–85% the intake of alcohol of animals subjected to deprivation periods and allowed alcohol reaccess. A marked inhibition of alcohol intake (−60%; $P < 0.001$) post deprivation was still observed 40 days after the single injection of MSCs.

Overall, studies presented constitute the first demonstration that an intracerebral administration of MSCs markedly inhibits the relapse-like consuming a drug of abuse.

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