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Effect of the combination of naltrexone and acamprosate on alcohol intake in mice

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Abstract Both naltrexone and acamprosate have been utilized clinically in recovering alcoholics with varying success. In the experiment reported here the combination of naltrexone and acamprosate was examined in a limited access alcohol model using *C57BL/6* mice to determine if there was evidence of additive or synergistic effects. The results of this experiment demonstrate that naltrexone, at the higher dose but not the lower dose, significantly reduced alcohol consumption. When combined with naltrexone, acamprosate reduced alcohol consumption across both doses of naltrexone. This effect was sensitive to both dose and number of days of exposure to the naltrexone/acamprosate combination.

Key words acamprosate, alcohol intake, *C57BL/6* mice, naltrexone.

INTRODUCTION

Alcohol dependence is a serious disease that results in financial, social, and medical problems, yet relapse prevention has not been easy to achieve. Treatments for relapse prevention of alcohol dependence are largely categorized into psychosocial treatments and pharmacotherapy. For pharmacotherapy, disulfiram has been available since the late 1940s; however, a placebo-controlled study of 605 alcohol dependent patients reported that only 20% of patients who were given disulfiram showed compliance.[1] Further, disulfiram treatment yielded only a modest advantage over placebo.[2] These data and others suggest that disulfiram's utility as a pharmacotherapeutic agent used to prevent relapse in alcohol dependence may be limited.

More recently, naltrexone, a non-specific opioid receptor antagonist, was shown to be effective in preventing relapse in a 12 week placebo-controlled study of 70 alcohol dependent patients. In that study, patients treated with naltrexone had a relapse rate of 23% while the placebo group had a 54% relapse rate.[3] These initial data were replicated[4] and led to Food and Drug Administration (FDA) approval of naltrexone for the treatment of alcoholism in 1994. Although approved by the FDA, the efficacy of naltrexone as a pharmacotherapy for alcoholism has not been fully determined. Compliance has been demonstrated to be critical for the clinical utility of naltrexone[5] and a recent study has suggested that there is no long-term difference between groups treated with either naltrexone or placebo.[6]

Another pharmacotherapeutic agent for the treatment of alcoholism is acamprosate, which is currently used in Europe[7] and is being evaluated by the FDA for use in the USA. Acamprosate is a structural analog of gamma-aminobutyric acid (GABA) and may have effects at both GABAergic and *N*-methyl-D-aspartate receptors.[8] A 12 month placebo-controlled study of 455 alcohol-dependent patients showed that treatment with acamprosate resulted in 230 abstinence days compared to 183 days for the control group. Moreover, 11.9% in the acamprosate treatment group and only 7.1% in the control group succeeded in continuous abstinence.[9]

The use of multiple drugs working at different sites has come to be a recognized treatment for diseases

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such as hypertension and diabetes.[10,11] In the case of alcoholism, some studies have shown that the combined administration of medications working on different neuronal systems is more effective in treating alcohol dependence than any one of the medications administered alone.[12,13] For example, in rats the combination of naltrexone, fluoxetine, and a thyrotropin-releasing hormone analog was more effective than any one of the drugs administered alone in reducing alcohol intake in mice.[12] In humans, both number of drinks per week and drinking days per week were significantly reduced when nalmefene, an opioid receptor blocker, was used in conjunction with sertraline, a serotonin reuptake inhibitor, compared to nalmefene treatment alone in alcohol dependent patients.[13]

Alcohol's direct and indirect effects are mediated by many different neurotransmitters within the central nervous system. The precise role of the endogenous opioid system in determining the motivational state underlying alcohol consumption is not yet well characterized. However, it is hypothesized that alcohol, acting either directly or indirectly with opioid receptors in the ventral tegmental area and nucleus accumbens, modulates the activity of the mesolimbic dopamine system.[14] In support of this view is evidence from microdialysis experiments showing that naltrexone blocks the alcohol-induced increase in dopamine in the nucleus accumbens of anesthetized rats.[15] In addition, in a recent experiment using microdialysis in awake alcohol self-administering rats, naltrexone concurrently reduced both alcohol consumption and extracellular dopamine levels in the nucleus accumbens.[16] Naltrexone's antagonism of the endogenous opioid system may then disrupt alcohol's reinforcing properties and reduce consumption by indirectly modulating mesolimbic dopamine pathways. Acamprosate, in contrast, is hypothesized to reduce alcohol consumption through different neural mechanisms. Acamprosate is hypothesized to increase activity of the inhibitory GABA receptors and decrease the activity in excitatory glutamate receptors.[17]

Given the questions remaining to be resolved about the clinical utility of both naltrexone and acamprosate for the treatment of alcoholism, the use of these drugs in combination may prove more efficacious than either drug alone. In the experiment reported here we examined the potential additive or synergistic effects of the combination of naltrexone and acamprosate on the alcohol intake of *C57BL/6* mice in a limited access procedure. This effect was examined across both a high and low dose of naltrexone, and naltrexone with acamprosate administered both immediately prior to limited access and 12 h prior to the start of limited access.

METHODS

Subjects

Forty-two 3-week-old male *C57BL/6* mice (Samtaco, Biokorea, Kyung-gi province, Korea) served as subjects in the present experiment. Upon arrival, animals were housed five per cage and allowed to adjust to the laboratory environment for 5 days. During that time, they had ad libitum access to water and food (Samyang Food, Seoul, Korea). Animals were maintained on a 12:12 h light : dark cycle. This research protocol was reviewed and approved by the Animal Research Committee of Medical Research Institute in Pusan National University Hospital.

Procedure

After the adjustment period, mice were forced to drink alcohol 10% (v/v) as their sole fluid choice for 7 days with food available 24 h a day. Following this, animals were individually housed and for the next 27 days (days 13–39) the mice were exposed to a limited access procedure in which they were given 2 h access to alcohol 10% (v/v) as their only fluid. For the remaining 22 h animals had ad libitum access to water. Food was available for 24 h. Alcohol consumption was measured before and after limited access to the nearest 0.001 g. Twenty-two h water and food intake was measured just prior to the start of the limited access session every day to the nearest 0.001 g. Fluid consumption was corrected for spillage.

Drug administration was carried out for 10 days (from days 40–49). On the first day (day 40), mice were divided into six groups randomly matched for consumption. These groups received the drugs as detailed in Table 1.

One-half of the daily dose of acamprosate was administered 12 h before the start of the limited access session and the other half 30 min before the start of the limited access session. Naltrexone was administered as

Table 1. Drug dosage

Group	*n*	Naltrexone dose (mg/kg)	Acamprosate dose (mg/kg)
1	7	0.025	None
2	6	0.025	50
3	6	0.025	200
4	7	1.0	None
5	7	1.0	50
6	7	1.0	200

a single dose 30 min before the start of the limited access session. For groups 1 and 4 (naltrexone only), an additional saline injection was given 12 h before the limited access session.

Drugs

Acamprosate (Hwan-In Pharm., Seoul, Korea) was mixed with saline, 0.9% and administered 12 h before the start of the limited access session and 30 min before the limited access session. Naltrexone (Je-Il Pharm., Seoul, Korea) was mixed with saline, 0.9% and administered 30 min before the start of the limited access session. All injections were given i.p. at a constant volume of 0.2 mL for each animal.

Statistical analysis

To minimize the effect of variability seen in daily intake, the mean of 2 day blocks was used. The baseline mean consisted of the 2 days preceding drug administration and the drug means consisted of the five 2 day blocks established across the period when drugs were injected. A repeated measures ANOVA (drug treatment group repeated across the five 2 day blocks) was used for group comparison of daily alcohol, water, and food intake. Simple effects tests were then used to analyze changes in consumption in individual drug treatment groups. When appropriate, Dunnett's post-hoc tests were used to determine differences in consumption between baseline control and individual 2 day drug treatment blocks. Also, Spjotvoll Stoline modification of Tukey's honestly significant difference (HSD) for groups with unequal n was used to compare alcohol consumption during each 2 day block between treatment groups.

RESULTS

Naltrexone at the high dose, but not at the low dose, significantly reduced alcohol consumption during the limited access session across the last four 2 day blocks. When acamprosate, at both the low and high dose, was administered together with the low dose of naltrexone, it significantly reduced alcohol consumption but only across the last 4 days of the experiment. Figure 1 shows alcohol consumption across the baseline and 10 drug days in 2 day blocks. A repeated measures ANOVA (six drug treatment groups repeated across six 2 day blocks) yielded a significant effect for block ($F_{5,170} = 33.701$, $P < 0.001$) with a significant group by block interaction ($F_{25,170} = 3.935$, $P < 0.001$). Simple effects tests of individual drug treatment groups revealed that all were significantly different. Subsequent post-hoc tests revealed that for drug group 1 (naltrexone 0.025 mg/kg alone), alcohol consumption during the third 2 day drug block was significantly higher than baseline with no other differences. For drug groups 2 and 3 (naltrexone 0.025 mg/kg + acamprosate 50 mg/kg and 100 mg/kg, respectively), alcohol consumption during the fourth and fifth 2 day drug blocks was significantly reduced when compared to baseline. For drug groups 4, 5 and 6 (naltrexone 1.0 mg/kg alone or + acamprosate 50 mg/kg and 100 mg/kg, respectively), alcohol consumption during the second, third, fourth and fifth 2 day drug blocks was significantly reduced compared to baseline.

Figure 2 shows this same ethanol consumption data expressed as difference scores (baseline minus each 2 day drug block divided by baseline) across the five 2 day blocks of the drug injection period. A repeated measures ANOVA (six treatment groups repeated across the five 2 day blocks) yielded a significant effect for group ($F_{5,34} = 17.944$, $P < 0.001$), a significant effect for

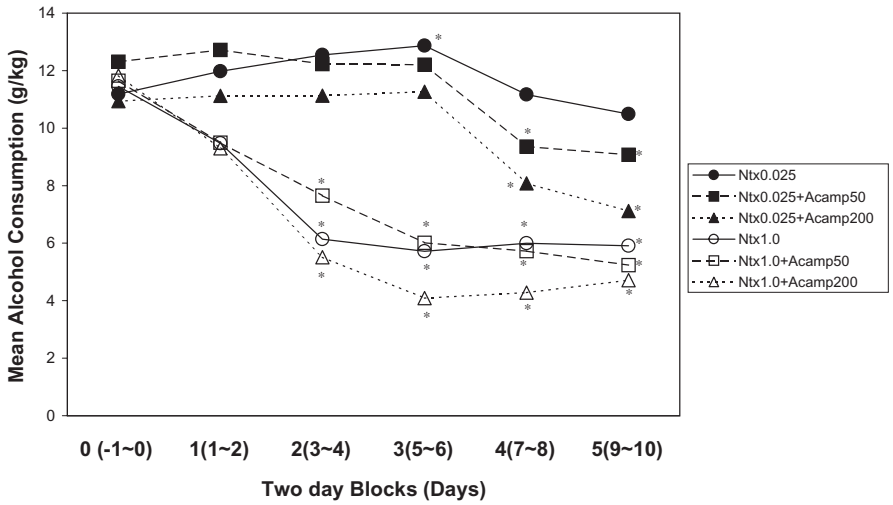

Figure 1. Mean ethanol consumption expressed as gm/kg collapsed into the mean of 2 day blocks across the last 2 days of baseline and the 10 days of drug treatment. Ntx, naltrexone; Acamp, acamprosate. $P < 0.001$, group by block, two-way ANOVA; $^*P < 0.05$ compared with baseline (block 0) by Tukey's post-hoc analysis.

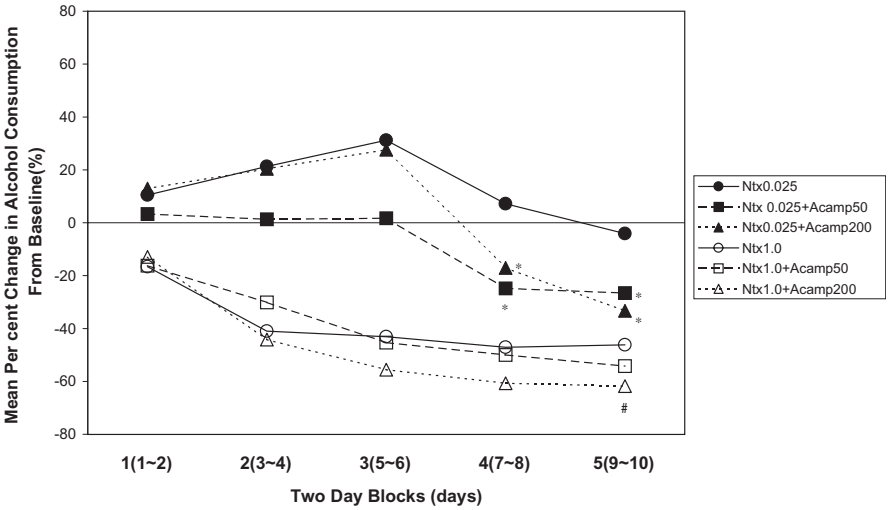

Figure 2. Mean difference score expressed as percent difference from baseline across the 10 days of drug treatment collapsed into the mean of 2 day blocks. Ntx, naltrexone; Acamp, acamprosate. $P < 0.001$, group by block, two-way ANOVA; $^{*}P < 0.05$ compared with Ntx0.025 by Tukey's post-hoc analysis; $^{\#}P < 0.05$ compared with Ntx1.0 or Ntx1.0 + Acamp50 by Tukey's post-hoc analysis.

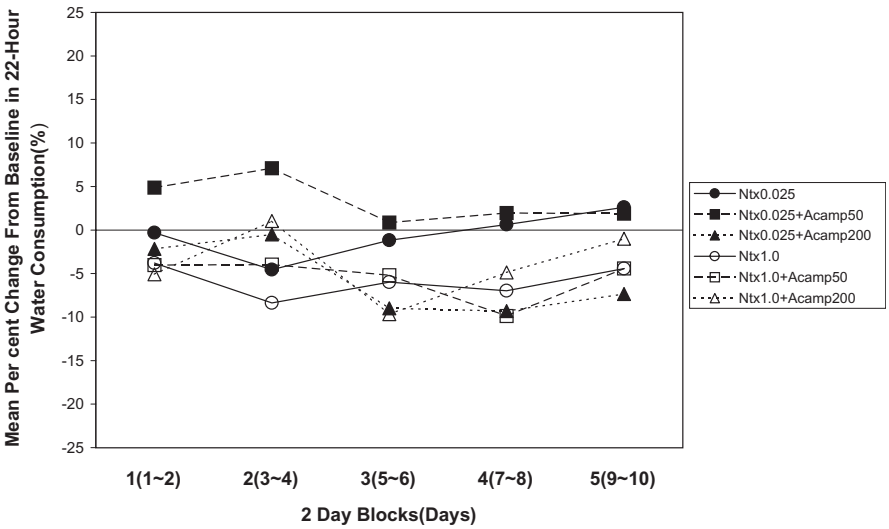

Figure 3. Mean 22 h water consumption expressed as mL collapsed into the mean of 2 day blocks across the last 2 days of baseline and the 10 days of drug treatment. Ntx, naltrexone; Acamp, acamprosate. No significance, two-way ANOVA.

blocks ($F_{4,136} = 44.952$, $P < 0.001$), and a significant group by block interaction ($F_{20,136} = 4.639$, $P < 0.001$). Subsequent simple effects tests of the difference scores revealed that the six drug treatment groups differed significantly across the last four 2 day drug blocks. Finally, post-hoc tests, Spjotvoll Stoline modification of Tukey HSD for groups with unequal *n*, were conducted to determine if the various drug treatments differentially suppressed ethanol consumption at each 2 day block. At all time points, groups 4, 5 and 6 had significantly greater suppression of ethanol consumption than groups 1, 2 or 3. Across the last two 2 day blocks groups 2 and 3 had significantly greater suppression of ethanol consumption than group 1 and finally, on the last 2 day block group 6 had significantly greater suppression of ethanol consumption than either group 4 or group 5.

Figure 3 shows 22 h water consumption across the baseline and 10 drug days in 2 day blocks. A repeated measures ANOVA (drug treatment group repeated across five 2 day blocks) revealed no significant differences. Figure 4 shows 24 h food consumption across the baseline and 10 drug days in 2 day blocks. A repeated measures ANOVA (drug treatment group repeated across five 2 day blocks) revealed no significant differences.

DISCCUSSION

The results of the experiment reported here demonstrate that a combination of naltrexone and acamprosate can reduce alcohol consumption in a limited access procedure. However, this additive effect is sensitive to the dose level of each drug and emerges only after the

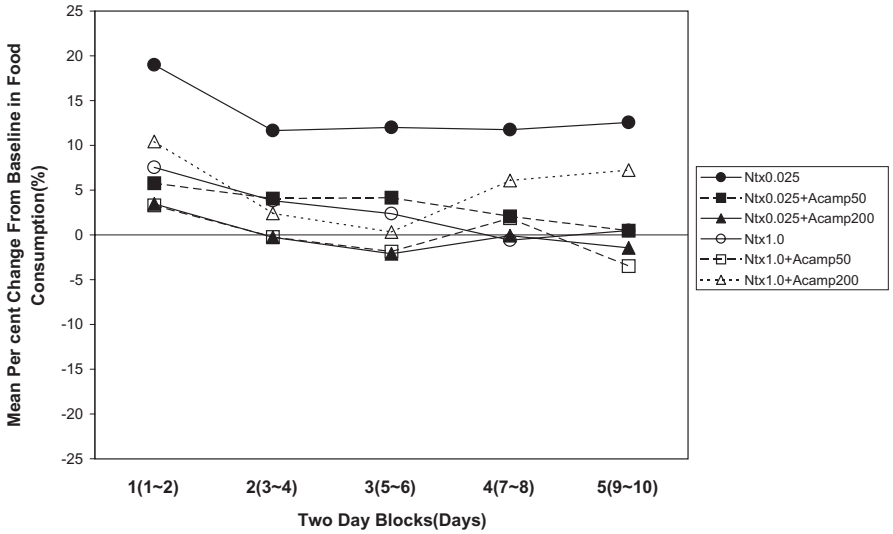

Figure 4. Mean 24 h food consumption expressed as g collapsed into the mean of 2 day blocks across the last 2 days of baseline and the 10 days of drug treatment. Ntx, naltrexone; Acamp, acamprosate. No significance, two-way ANOVA.

mice have been exposed to acamprosate and alcohol for a number of days. The suppressive effect of naltrexone and acamprosate appears to be selective for alcohol because there was no effect of either drug or their combination on food or water consumption.

While naltrexone at the higher dose (1.0 mg/kg) significantly reduced alcohol consumption when administered alone, at the lower dose (0.025 mg/kg) naltrexone not only failed to suppress alcohol consumption but led to increases in alcohol consumption including a significant increase across one 2 day block. The goal of the present study was to evaluate the potential for an additive or synergistic effect of the addition of acamprosate to a pharmacotherapy that included naltrexone, therefore acamprosate was not examined in isolation. However, some preclinical and clinical studies have shown that acamprosate, when administered alone, were effective in reducing alcohol consumption,[18,19] but others were not.[20,21]

The data in Fig. 2 showing alcohol consumption expressed as a difference score from baseline demonstrate that acamprosate, at either the high or low dose tested, significantly suppressed alcohol consumption across the last 4 days of the experiment, when administered together with either the low or high dose of naltrexone. These data suggest that acamprosate's effect emerges only after the mouse has had some extended opportunity to experience the effects of both alcohol and acamprosate together. This finding is comparable to other reports in the literature.[22,23]

The finding that group 2 (naltrexone 0.025 mg/kg + acamprosate 50 mg/kg) and group 3 (naltrexone 0.025 mg/kg + acamprosate 200 mg/kg) produced significantly greater suppression of alcohol consumption than group 1 (naltrexone 0.025 mg/kg alone) across the last 4 days of the experiment demonstrates an additive effect for both the low and high doses of acamprosate when they are added to a subclinical dose of naltrexone. Group 4 (naltrexone 1.0 mg/kg alone) produced significantly greater suppression of alcohol consumption across the last 8 days of the experiment when compared to groups 1, 2 and 3, demonstrating a dose effect for naltrexone. Only group 6 (naltrexone 1.0 mg/kg + acamprosate 200 mg/kg) produced significantly greater suppression of alcohol consumption when compared to group 4 (naltrexone 1.0 mg/kg alone). When taken together with the results for group 2, these data suggest that the higher dose of naltrexone may have produced a floor effect that masked any additive effect of the lower dose of acamprosate.

The difference between the results presented here and those of Heyser *et al.* and Stromberg *et al.* related to the effect of acamprosate on baseline alcohol consumption[20,21] is probably attributable to procedural differences among the experiments. Key among those differences is the number of pairings of acamprosate and alcohol. The experimental design used by Heyser *et al.* exposed rats to 5 days of a single injection of acamprosate, 25, 100 and 200 mg/kg, 30 min before the opportunity to consume alcohol using an operant lever press procedure,[20] while the design of Stromberg *et al.* exposed rats to 4 days of acamprosate 50 and 200 mg/kg administered 30 min before limited access drinking in the home cage.[21] Although not significant, the trend of the Heyser *et al.* data for the highest dose of acamprosate (200 mg/kg) showed that repeated exposure to acamprosate produced a linear decrease in alcohol consumption.[20] The effects of acamprosate in the experiment reported here were not apparent until the rats had 7 days of exposure to

acamprosate administered twice daily. This is also consistent with data provided by Gewiss *et al*., who reported a decrease in alcohol preference dependent on dose following either 11 or 13 days of drug exposure.[24]

The present results suggest that alcohol consumption in the limited access model is mediated by more than one underlying neural system. Acamprosate is hypothesized to exert its influence on alcohol consumption through antagonist effects at the glutamate receptor, which has been hypothesized to reduce craving for alcohol, which emanates from neuronal hyperexcitability produced by alcohol withdrawal.[8,25] Acamprosate may function to reduce alcohol consumption by attenuating neuronal hyperexcitability, thereby attenuating the negative reinforcing properties of alcohol. In contrast, there are *N*-methyl-D-aspartate/glutamate receptors in the nucleus accumbens receiving input from the amygdala, hippocampus,[26] prefrontal cortex[27] and ventral tegmental area.[28] These receptors have been shown to modulate dopaminergic activity in the nucleus accumbens,[29,30] and could therefore disrupt the positive reinforcing properties of alcohol as well. Naltrexone, a non-selective opioid antagonist, achieves its effects through *mu* and perhaps *delta* opioid receptor subtypes.[31,32] These opioid systems indirectly modulate alcohol-induced dopaminergic activity, which is hypothesized to underlie alcohol's reinforcing properties[33] through *delta* opioid receptors in the nucleus accumbens,[15,16,34] and through *mu* opioid receptors in the ventral tegmental area.[35]

There was no evidence that the administration of either acamprosate, twice per day, or naltrexone, 30 min before limited access, reduced consumption of either food or water across the 24 h period. These findings suggest that this particular combination of drugs is selective for alcohol rather than producing a more general disruption in appetitive behavior.

In summary the findings from the present experiment add to and extend the literature by showing that acamprosate, when combined with naltrexone, can produce significantly greater suppression of alcohol consumption than naltrexone given alone. This additive effect is not apparent immediately but only after the rat has had the opportunity to experience alcohol and acamprosate together for 8 days.

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