Synergistic action of naltrexone plus topiramate on ethanol self-administration in C57BL/6 mice and associated neurochemical changes

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OBJECTIVES OF THE STUDY

Naltrexone has demonstrated a high efficacy to reduce ethanol consumption and relapse, being one of the most employed medication for alcohol dependence treatment (1,2). In the last years, the coadministration of topiramate with naltrexone has emerged as a very useful drug for alcohol dependence management (3,4).

The first objective of the present study was to evaluate if the combination of naltrexone and topiramate, in comparison with monotherapy, leads to a higher reduction of ethanol consumption employing a self-administration procedure.

The second objective was to analyze if naltrexone, topiramate or their combination induce different gene expression changes in key targets related with alcohol dependence.

MATERIAL AND METHODS

Animals
Male C57BL/6Jad mice (age 8-10 weeks, 20-25 g) were purchased from Harlan (Barcelona, Spain). Mice were housed under controlled conditions (23 ± 2°C, 12 h light:12 h dark). All experiments were approved by the institutional Animal Care Committee.

Drugs
Ethanol 6% v/v (equivalent to 7.5% v/v) was dissolved in distilled water. Topiramate/TPM (25 mg/kg, p.o.) - Topamax® and Naltrexone/NTX (0.7 mg/kg, p.o. - Anexivid®) were dissolved in distilled water and administrated orally or in combination 1 hour prior testing, during the last phase of self-administration procedure.

Behavioral analyses
The evaluation of the ethanol self-administration was carried out in twelve modular open chambers (Farina, Barcelona, Spain). The experiment was divided in four phases: training, substitution, postsubstitution and based ethanol 6% consumption and pharmacological treatment effect on ethanol 6% consumption (Figure 1A). The procedure to evaluate water self-administration consisted of two phases: training and pharmacological treatment effect on water consumption (Figure 1B).

Neurochemical analyses
Mice were decapitated and brains were removed from the skull and frozen over dry ice. Total RNA was isolated from nucleus accumbens (NAcc) and the ventral tegmental area (VTA) microdissections (5,6) using TRI reagent (Applied Biosystems) and subsequently reverse transcribed to cDNA. Quantitative analysis of the relative abundance of µ-opioid receptor (ρOR), cannabinoid CB1 receptor and tyrosine hydroxylase gene expression was performed by real-time PCR (Applied Biosystems). The reference gene used was 18S rRNA, detected using Taqman ribosomal RNA control reagents. Briefly, data for each target gene were normalized to the endogenous reference gene, and the fold change in target gene mRNA abundance was determined using the 2^-ΔΔCt method (7).

Statistical analyses
Statistical analyses were performed using one-way or two-way analysis of variance followed by the Student-Newman-Keuls test. Differences were considered significant if the probability of error was less than 5%. Significance v3.11 software was used.

1. Experimental Design

2. Ethanol Self-Administration - Evolution Before Pharmacological Treatment

3. Effect of Naltrexone and/or Topiramate on Ethanol Self-Administration

4. Effect of Naltrexone and/or Topiramate on Water Self-Administration

5. Gene expression

REFERENCES

SUMMARY AND CONCLUSIONS

• The co-administration of naltrexone and topiramate significantly reduced the ethanol consumption and motivation to a greater extent compared to any of the drugs given separately.

• The effects observed in the ethanol self-administration procedure were specific since treatment with naltrexone, topiramate or its association did not modify the motivation toward a neutral stimulus such as water.

• The co-administration of naltrexone and topiramate produced a more pronounced reduction of the gene expression of µ-opioid receptor, cannabinoid CB1 receptor and tyrosine hydroxylase, compared to any of the drugs given alone.

• Taken together, these findings suggest that the combination of both drugs may deserve further exploration for the treatment of problems related with alcohol consumption.