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**Research Report**

# Red wine but not ethanol at low doses can protect against the toxicity of methamphetamine

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**ABSTRACT**

The goal of this study was twofold: (a) to search for possible interactive effects between two common drugs of abuse, ethanol and methamphetamine. b) To inquire whether any effects of ethanol could be replicated using an equivalent amount of ethanol in the form of red wine. Adult male C57/6 N mice received 2% ethanol for 8 weeks in drinking water or red wine diluted to yield the same ethanol content. On the 9th week animals received multiple injections of methamphetamine (4 × 10 mg/kg, ip, every 2 h). They were then sacrificed 72 h after treatment. Methamphetamine produced a significant depletion of dopamine and DOPAC in the striatum. Treatment with both ethanol and methamphetamine led to a reduction of striatal dopamine and DOPAC that were both non-significantly greater than that observed with methamphetamine alone. Alcohol alone produced no changes in the striatal content of dopamine or its metabolite, DOPAC. These data suggest that low doses of alcohol potentiate methamphetamine-induced neurotoxicity in mice and that this combination may be especially detrimental to the brain. However, an equivalent dose of ethanol in the form of red wine actually partially protected against methamphetamine-induced depletion of dopamine and DOPAC in red wine treated mice. This implies the presence of other agents in red wine, which may mitigate the toxicity of methamphetamine.

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**1. Introduction**

Alcohol has been in common use for several millennia and has been widely misused. The relationship between alcohol consumption with various diseases has been explored for decades. In early seventies several French scientists published a series of epidemiological studies showing the beneficial effects of wine consumption and the lowering of heart disease what they described as “French Paradox” (Das et al., 1999). Most, biochemical studies in the literature concerning alcohol

have used moderate to high doses to better understand its mechanism of action. There are some reports suggesting that at a low concentration, alcohol may be beneficial by detoxification of oxidant free radicals (Constant, 1997). However, at high concentrations it can act as a pro-oxidant by various means including induction of the mixed function oxidase CYP2E1 (Wu and Cederbaum, 2005), and formation of peroxynitrite (Yang et al., 2008).

In the scientific literature studies generally explore the effect of a single drug on a given system. However, in the real

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world, drug abusers often use several drugs in conjunction. Alcohol is commonly used together with other drugs of abuse (Kenna et al., 2007a,b). Methamphetamine is an increasingly major drug of abuse (Klein-Schwartz & McGrath, 2003; Yacoubian et al., 2003). This agent also causes neurotoxicity in rodents and nonhuman primates (Krasnova and Cadet, 2009; Yamamoto et al., 2010). It produces its neurotoxicity by depleting the DA concentration, the number of high affinity DA uptake sites and in the activity of tyrosine hydroxylase (TH) in striatum (Villemagne et al., 1998; Volz et al., 2007). Methamphetamine-induced neurotoxicity may in part be due to the generation of pro-oxidant free radicals (Ali et al., 1993; Itzak and Ali, 2006). Therefore, the present study was designed to determine whether extended low level exposure to either pure ethanol or ethanol in the form of red wine could attenuate or potentiate METH-induced neurotoxicity.

## 2. Results

The volume of fluid consumed by each mouse group did not differ significantly and was 4.2–4.5 ml/mouse/day. Thus in the case of the mice drinking diluted ethanol or red wine, daily ethanol consumption was  $2.7 \pm 0.2$  g/kg body weight. The body weights of the mice in each group were unaffected by treatment. Chronic exposure to alcohol produced no significant changes in dopamine or DOPAC concentration in the striatum. As expected, methamphetamine produced a significant depletion of DA and its metabolite DOPAC in the striatum. When ethanol was present in the drinking water, the methamphetamine-induced depletion was not significantly altered although there was a non-significantly larger effect of methamphetamine on both dopamine and DOPAC levels. When the same dose of ethanol was administered in the form of red wine, a significant protective effect upon the catecholamine depletion was found (Figs. 1, 2). No effects of ethanol or red wine were found on levels of serotonin or HIAA, indices of serotonergic integrity (data not shown).

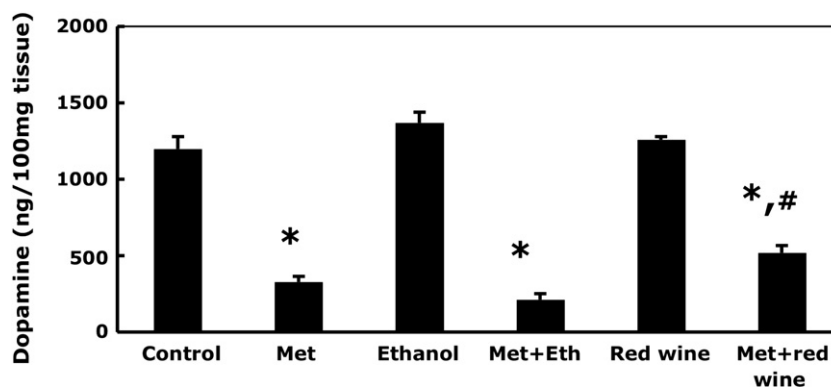
## 3. Discussion

These findings imply that there are components of red wine that can act in a neuroprotective manner if administered prior to methamphetamine dosing. This active component is not ethanol. However, it has previously been reported that high but not low doses of ethanol can attenuate methamphetamine-induced dopamine depletion (Yu et al., 2002).

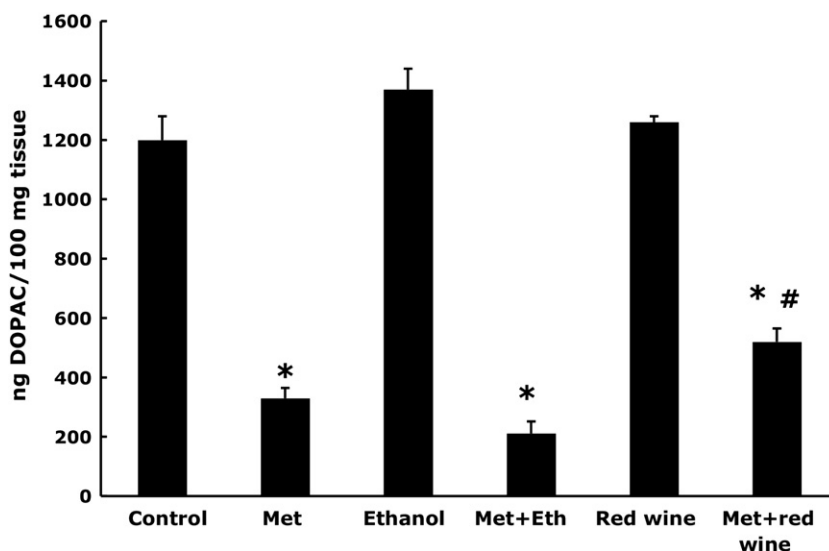
Red wine contains a large range of polyphenolic and flavonoid compounds (Croft, 1998; Sun et al., 2002) and it remains to be determined whether the protective agent is related to resveratrol. Resveratrol can attenuate the effect of acute MPTP treatment, in depleting dopamine and its metabolites (Blanchet et al., 2008) and can retard the development of Parkinsonian symptoms following administration of 6-hydroxydopamine (Jin et al., 2008). Direct comparison with our findings is difficult since the study of Blanchet employed doses of resveratrol several orders of magnitude greater than those found in red wine. Furthermore, red wine contains a variety of flavonoids in addition to resveratrol. In order to further define the potentially protective properties of physiological levels of resveratrol, it would be useful to compare the effects of red wine with those of white wine and of red grape juice.

The dopamine system is likely to be the common target of both methamphetamine and ethanol toxicity and its disruption may form the basis of addiction (Rothman et al., 2000). Dopaminergic neurons may also form a significant site of action for the neuroprotective effects of resveratrol. In fact, resveratrol protects dopamine neurons from a wide range of neurotoxic agents (Okawara et al., 2007). That the effects of red wine and an aqueous solution with the same ethanol content are markedly different are emphasized by two reports that red wine is actually protective against ethanol-induced oxidative stress in rat liver (Kasdallah-Grissa et al., 2007; Assunção et al., 2009).

This report underscores the need for a polypharmacological approach in the study of drugs of abuse. While this makes the elucidation of underlying mechanisms more difficult, it can uncover unexpected drug interactions, which are most relevant to the societal clinical needs surrounding drug abuse.



**Fig. 1** – Effect of ethanol or red wine upon methamphetamine-induced dopamine depletion in the mouse striatum. Groups of mice ( $n=16$ ) received water (control), 2% ethanol or 2% ethanol as diluted red wine. After 9 weeks half of each group was subjected to an acute methamphetamine challenge. \*Differs from value for corresponding animals not injected with methamphetamine. #Differs from value for water-drinking mice injected with methamphetamine ( $p < 0.05$ ).



**Fig. 2 – Effect of ethanol or red wine upon methamphetamine-induced DOPAC depletion in the mouse striatum. Groups of mice ( $n=16$ ) received water (control), 2% ethanol or 2% ethanol as diluted red wine. After 9 weeks half of each group was subjected to an acute methamphetamine challenge. \*Differs from value for corresponding animals not injected with methamphetamine. #Differs from value for water-drinking mice injected with methamphetamine ( $p<0.05$ ).**

#### 4. Experimental procedures

Adult C57/B6n male mice were used and were housed 4 per cage under controlled conditions. The mice were given 2% ethanol in drinking water, either as native ethanol or as red wine appropriately diluted. A control group received water alone. Food was provided ad libitum. The ethanol exposure continued for 8 weeks. At the beginning of 9th week mice were injected four times with 10 mg/kg methamphetamine (METH) or an equivalent volume of saline intraperitoneally (ip) ( $N=8$ ) at 2-h intervals and were sacrificed 72 h later by cervical dislocation. Brains were rapidly removed and the striatum dissected out and stored at  $-70^{\circ}\text{C}$  for monoamine assay. Concentrations of DA, 5-HT and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) were quantitated by a modified method of high performance liquid chromatography (HPLC) combined with electrochemical detection. Briefly, each striatum was weighed in a measured volume (20 % w/v) of 0.2 N perchloric acid containing 100 ng/mg of the internal standard 3,4-dihydroxybenzylamine (DHBA) was added. The tissue was then disrupted by ultrasonication, centrifuged (15,000g; 7 min), and 150  $\mu\text{l}$  of the supernatant was removed and filtered through 0.2  $\mu\text{m}$  Nylon-66 microfilter (MF-1 centrifugal filter, Bioanalytical System (BAS), W. Lafayette, IN). Aliquots of 25  $\mu\text{l}$  representing 2.5 mg of brain tissue were injected directly onto the HPLC/EC system. The HPLC analytical system included a Waters Associates Model 510 liquid chromatographic pump (Milford, MA), a Rheodyne Model 7125 injector (Rheodyne, Inc., Cotati, CA), a Supelco Supelcosil LC-18, 3  $\mu\text{m}$  (7.5 cm  $\times$  4.6 mm) analytical column, a LC-4B amperometric detector and LC-17 oxidative flow cell (BAS) consisting of a glassy carbon electrode (TL-5) versus Ag-AgCl reference electrode maintained at a potential of 0.75 V. The mobile phase consisted of

0.07 M potassium phosphate, pH 3.0, 8% methanol and an ion pairing reagent of 1.02 Mm 1-heptane sulfonic acid. Chromatograms were recorded and integrated on a Perkin-Elmer LCI-100 integrator (Perkin-Elmer Corp., Norwalk, CT) (Ali et al., 1993). The concentration of DA, DOPAC and 5-HIAA were calculated using a standard curve. The standard curves were generated by determining in triplicate the ratio between 3 different known amounts of each amine or its metabolite and a constant amount of internal standard.

Neurotransmitter concentration data were analyzed by analysis of variance (ANOVA), followed where appropriate by Duncan's multiple range test (Duncan, 1955). A value of  $p<0.05$  was taken as significant.

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