PHARMACODYNAMIC RESPONSE TO Ro15-4513 IN ETHANOL INTOXICATED DOGS

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SUMMARY

The interaction of Ro15-4513 (5 mg/kg) with ethanol (3 g/kg, 60%w/v bolus) in dogs was investigated. Ro15-4513 challenge 120 minutes after a single ethanol dose had no significant effect on blood ethanol concentration or heart rate. In the same experiment, (1) blood acetaldehyde concentration was elevated to more than double the control value (vehicle only, no Ro15-4513), and (2) systolic blood pressure decreased to less than 60% of control. Further investigation revealed: (1) after Ro15-4513, area under the blood acetaldehyde vs time curve was more than twofold greater ($p=0.0006$) than control, and the area under the systolic blood pressure vs time curve was 76% ($p=0.0027$) of control. Based on these results, we propose that an inter-relationship exists between Ro15-4513, blood acetaldehyde concentration, and systolic blood pressure in ethanol intoxicated dogs.

KEY WORDS

Ro15-4513, ethanol, alcohol, drug interaction

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INTRODUCTION

Since Suzdak's early report that Ro15-4513 blocked ethanol intoxication in rats /1/, over 300 papers dealing with widely varying aspects of Ro15-4513 have appeared. Understandably, Suzdak's results aroused interest in possible use of Ro15-4513 as pharmacotherapy for ethanol intoxication. Numerous studies measured blood ethanol after Ro15-4513 administration to ethanol challenged subjects, but no study found that Ro15-4513 had a significant effect on blood ethanol. It is now generally accepted that Ro15-4513 exerts its ethanol antagonizing activities through its role as a benzodiazepine receptor inverse agonist, and not through any direct effects on ethanol (see the review by Jackson and Nutt /2/ and references therein).

Acetaldehyde, the first oxidation product of ethanol metabolism, possesses significant pharmacological properties independent of those properties normally associated with ethanol /3-5/. However, no report has appeared that considered association of Ro15-4513 administration and blood acetaldehyde levels. The present investigation sought to ascertain association between administration of Ro15-4513 and blood acetaldehyde values in ethanol intoxicated dogs. The scope of the investigation was limited to a single dose of Ro15-4513 and a relatively high ethanol dose in dogs, with measurement of blood ethanol and blood acetaldehyde concentrations, heart rate, and systolic blood pressure.

MATERIALS AND METHODS

Ethanol and Ro15-4513 dosing

With institutional approval, two groups of five mongrel dogs each (body weights=16-30 kg; mean wt.=22.4 kg) were administered a single bolus ethanol dose (3 g ethanol/kg, 60% w/v in drinking water, via orogastric tube). At 120 minutes after the ethanol dose, the experimental group received Ro15-4513 (5 mg/kg in 1% Tween 80, i.v.). The control group received only the 1% Tween 80 vehicle but was otherwise treated identically to the experimental group. Ro15-4513 was a kind gift of Dr. Steven Paul of the National Institute of Mental Health.

Blood ethanol and acetaldehyde

Each dog was shaved and swabbed with Betadine solution at the venipuncture site prior to any blood sampling. Blood (3 ml) was taken from a catheter placed in the saphenous vein for the duration (8 hours) of the experiment. A single blood sample (3 ml) was taken from each dog before the ethanol dose (0 minutes). Additional blood samples were taken at 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 180, 240, 300, and 360 minutes after the ethanol dose. Blood was mixed with 7.5 mg solid sodium fluoride and 6.0 mg potassium oxalate to prevent coagulation. Samples and reagents were maintained on ice unless otherwise noted. Standards were prepared by mixing 0.9 ml of anticoagulated blood with 0.1 ml of acetaldehyde (Fisher Scientific, Atlanta, GA) solution or ethanol (Aaper Alcohol Co., Shelbyville, KY) solution yielding 0, 2, 4, 6, 10, 20, or 40 μM acetaldehyde, or 0, 2, 6, 12.5, 25, 60, or 100 mM ethanol. Each standard or sample (1 ml) was then mixed with 0.5 ml of perchloric acid (1 N PCA, 20 mM thiourea) (PCA) and centrifuged. Supernatant of 0.6 ml was sealed in glass vials for head-space analysis using a Hewlett-Packard 5890 II gas chromatograph (GC) utilizing a flame ionization detector (FID) with a 6 foot x 1/4 inch column packed with Tenax GC along with a Hewlett-Packard 19395 autosampler. Samples were incubated in sealed GC vials at 60°C for ten minutes prior to GC headspace sampling and during GC headspace sampling. Additional ethanol and acetaldehyde analysis details are given by Whitmire *et al.* /14/ and Fukunaga *et al.* /15/ and references cited therein.

Heart rate and indirect systolic blood pressure (blood pressure) were monitored according to Kirk and Bistner /6/ using a Dynamap Doppler sphygmomanometer. Heart rate and blood pressure values were recorded at 0, 12, 22, 32, 42, 53, 63, 84, 103, 120, 135, 145, 155, 165, 175, 180, 244, 304, and 363 minutes.

Differences between means were evaluated using Student's t-test.

RESULTS

Observations

In the control group, after the ethanol dose, each dog became calm and slept lightly. However, on stimulation by a technician each dog was playful and nearly fully stable during walking.

In the experimental group within five minutes after injection with Ro15-4513, each dog turned his head upward off the examination table and vocalized loudly. Each dog's eyes opened widely with total dilatation of the pupils. The dogs did not respond at all to verbal or tactile stimulation by the technician and were completely unable to stand. This aroused but unresponsive state lasted approximately 20 minutes, after which each dog slept heavily and was not responsive to stimulation. The period of heavy sleep lasted up to six hours.

Ethanol, acetaldehyde, heart rate, and blood pressure

Control and experimental maximum mean blood ethanol concentration values were 71.0±4.6 mM and 75.8±5.8 mM respectively (mean ± S.E.M.).

Percentage change was computed for blood ethanol and acetaldehyde concentrations, heart rate, and blood pressure for the control and experimental groups (Table 1). Percentage change was computed as the difference in each measured variable over the period

TABLE 1

Percentage change in ethanol metabolites and vital signs

Measured variable	Mean percent change		
	Control	Ro15-4513	p-value
Blood acetaldehyde concentration	-22.3 ± 7.0	39.3 + 10.1	0.01
Blood ethanol concentration	-6.7 ± 3.1	9.6 + 6.3	0.11
Systolic blood pressure	2.8 ± 3.9	-30.2 ± 5.2	0.01
Heart rate	0.3 ± 3.0	-1.2 ± 11.0	0.90

Five dogs were dosed with ethanol (3 g/kg, 60% w/v) at time zero. Experimental dogs were given Ro 15-4513 (5 mg/kg i.v. in 1% Tween-80) 120 minutes after the ethanol dose. Control dogs received the Tween-80 vehicle with no Ro 15-4513, but were otherwise treated identically to the experimental dogs. The above values are mean percentage changes in the measured variables for each group of five dogs. Percentage change was computed as the difference in the measured variable over the period from 180 minutes to 120 minutes, divided by the 120 minute value. Values shown are means ± S.E.M. The p-values are from t-tests of the means of control and experimental groups, with $p<0.05$ taken as significant.

from just prior to the Ro15-4513 injection at 120 minutes, to one hour later at 180 minutes, divided by the pre-injection value at 120 minutes.

Mean acetaldehyde concentration decreased (-22.3%) in the control group but increased (+39.3%, p=0.01) in the experimental group. Mean blood pressure increased only slightly in the control group (+2.8%), but decreased (-30.2%, p=0.01) in the experimental group. Differences in the mean percentage change between the control and experimental groups were not significant for blood ethanol concentration nor heart rate. Based on these results (Table 1), acetaldehyde concentration and blood pressure were investigated further.

Mean blood acetaldehyde concentration and mean blood pressure for control and experimental groups are plotted versus time in Figures 1 and 2 respectively. After Ro15-4513 injection at 120 minutes, the mean blood acetaldehyde concentration in the experimental group

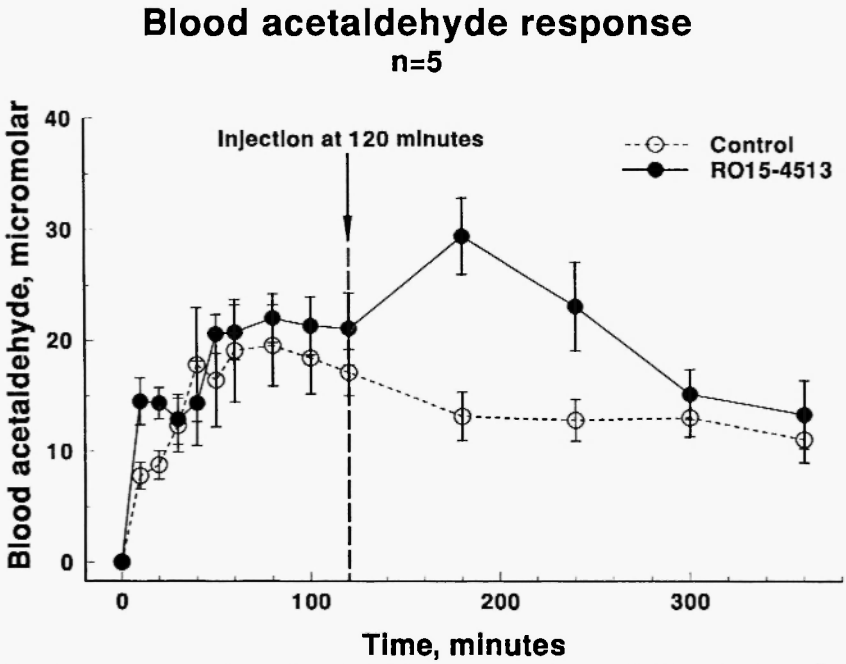

Fig. 1: Response of blood acetaldehdye concentration to Ro15-4513 (5 mg/kg i.v.) in ethanol intoxicated dogs. Dogs received a single bolus of ethanol (3 g/kg, 60% w/v, p.o.) at 0 minutes. Open circles with a dashed line indicate the control group, filled circles with a solid line indicate the experimental group. Error bars indicate standard error of the mean.

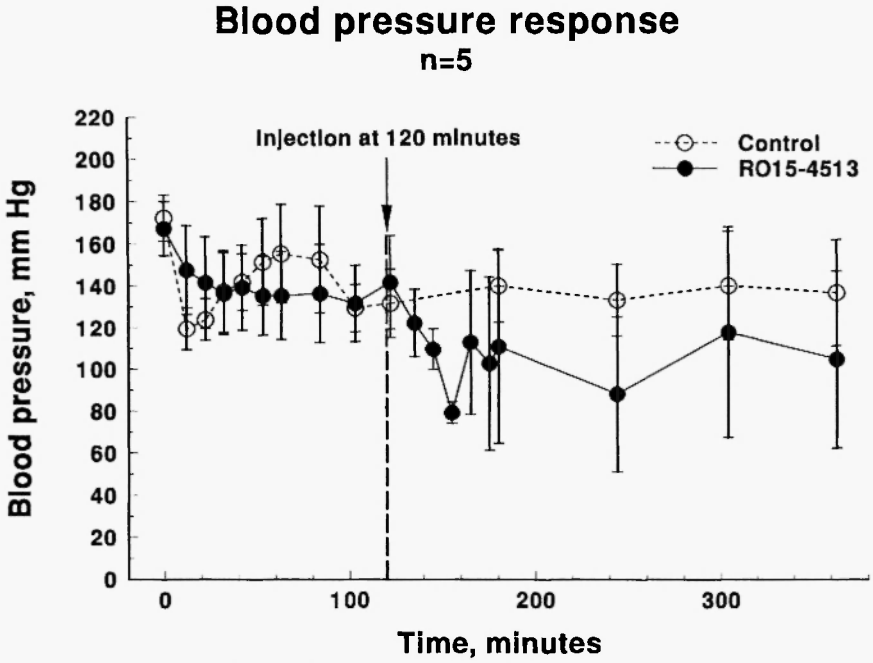

Fig. 2: Response of systolic blood pressure to Ro15-4513 (5 mg/kg i.v.) in ethanol intoxicated dogs. Dogs received a single bolus of ethanol (3 g/kg, 60% w/v, p.o.) at 0 minutes. Open circles with a dashed line indicate the control group, filled circles with a solid line indicate the experimental group. Error bars indicate standard error of the mean.

increased to approximately twice the control value at 180 minutes, and then there was a downward trend until the experimental and control values were essentially the same at 360 minutes. Similarly, after Ro15-4513 injection at 120 minutes, the mean blood pressure of the experimental group initially decreased to about one-half of the control value, and remained depressed below control values thereafter.

Area under the curve (AUC) was computed for pre-injection (0-120 minute) and post-injection (120-360 minute) segments of the blood acetaldehyde concentration vs time curve, and the blood pressure vs time curve (Table 2). For control and experimental groups, there was no significant difference between pre-injection AUCs for blood acetaldehyde ($p=0.87$) or blood pressure ($p=0.41$); significant differences were obtained for post-injection AUCs for both blood acetaldehyde ($p=0.0006$) and blood pressure ($p=0.0027$).

TABLE 2

Area under the curve (AUC) for blood acetaldehyde and blood pressure

	Control	Ro15-4513	p-value
Pre-injection period (0-120 min)			
Blood acetaldehyde, μM-min	1950 + 260	1900 ± 73	0.87
Blood pressure, mm Hg-min	18278 + 2512	17548 ± 3144	0.41
Post-injection period (120-360 min)			
Blood acetaldehyde, μM-min	2165 ± 322	4704 ± 534	0.0006
Blood pressure, mm Hg-min	33329 + 5987	25042 ± 4821	0.0027

Five dogs were dosed with ethanol (3 g/kg, 60% w/v) at time zero. Experimental dogs were given Ro 15-4513 (5 mg/kg i.v. in 1% Tween-80) 120 minutes after the ethanol dose. Control dogs received the Tween-80 vehicle with no Ro 15-4513, but were otherwise treated identically to the experimental dogs. The above are AUC values for blood acetaldehyde vs time in terms of μM-minutes, and blood pressure vs time in terms of mm Hg-minutes. Values shown are means ± S.E.M. The p-values are from t-tests of the means of control and experimental groups, with $p<0.05$ taken as significant.

DISCUSSION

Observations

Ro15-4513 is reported to have anxiogenic and proconvulsant effects as a result of its inverse agonist action at the benzodiazepine receptor in the brain /2/. The behavioral effects observed immediately after intravenous administration of Ro15-44513 to the ethanol intoxicated dogs in this study are consistent with this mechanism of action.

Blood ethanol curve

Similar to numerous other investigators /2/, we concluded that Ro15-4513 caused no significant changes in the blood ethanol curve.

Heart rate

We found that Ro15-4513 administration had no significant effect on heart rate of ethanol intoxicated dogs. This finding is consistent with that of Lerner *et al.* who found Ro15-4513 had no effect on heart rate in ethanol challenged rats /13/.

Blood acetaldehyde

Our finding that blood acetaldehyde concentrations were elevated over control values could possibly have resulted from three mechanisms. Ro15-4513 administration may have caused: (1) a transient net increase in alcohol dehydrogenase (ADH) activity, (2) a transient net decrease in aldehyde dehydrogenase activity, or (3) a release of otherwise sequestered acetaldehyde.

Although significant quantities of acetaldehyde could possibly be protein-bound during ethanol metabolism, we found no basis with which to support mechanism (3) listed above. Similarly, we have found no evidence that Ro15-4513 inhibits acetaldehyde dehydrogenase *in vivo* as proposed by mechanism (2). However, based on literature references, the following explanation may provide support for mechanism (1).

Langeland *et al.* showed that Ro15-4513, in competition with ethanol, was a reversible inhibitor of horse liver alcohol dehydrogenase *in vitro* /7/. According to Langeland, Ro15-4513 inhibition of ADH resulted from Ro15-4513 interaction with the zinc center of ADH. However, Langeland used only 8 mM ethanol for *in vitro* assay. Our *in vivo* experiments with dogs resulted in blood ethanol concentrations nearly 10-fold greater than Langeland's *in vitro* assay. Therefore, it seems possible that the relatively high ethanol concentrations (~80 mM) used in the present study diminished the ability of Ro15-4513 to compete with ethanol (8 mM) for the zinc center of ADH described by Langeland. Moreover, Theorell demonstrated that compounds with an imidazole ring can form ADH-NADH-imidazole complexes at relatively high ethanol concentrations /8/. Theorell found that the ADH-NADH-imidazole complex is more labile than the endogenous

ADH-NADH complex formed naturally, thus allowing more rapid dissociation of NADH from ADH. ADH-NADH dissociation is known to limit ADH activity; thus, an increase in the velocity of the rate limiting ADH-NADH dissociation step, via the more labile ADH-NADH-imidazole complex, would tend to result in a net increase in ADH activity. The Ro15-4513 structure (Fig. 3) contains an imidazole ring (Fig. 4) which might possibly be made available for participation in the mechanism described by Theorell via metabolism of Ro15-4513.

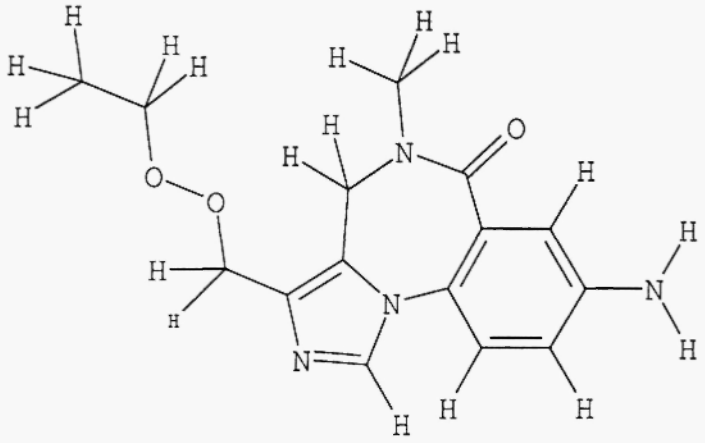

Fig. 3: Structure of Ro15-4513.

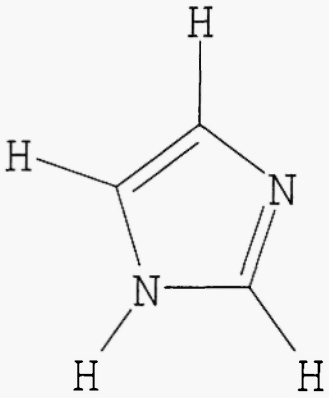

Fig. 4: Structure of imidazole.

If ADH activation accelerated ethanol conversion to yield increased blood acetaldehyde the increased acetaldehyde would likely be detectable on the blood acetaldehyde curve since control blood acetalde-hyde values are typically very low (10-15 μM). For example, if ADH activation caused a transient increase of 10-15 μM acetalde-hyde, this would result in blood acetaldehyde values approximately twofold greater than normal baseline control values of 10-15 μM; this level of blood acetaldehyde is easily detected using the headspace GC technique described above. However, any acetaldehyde increase would result in a stoichiometric decrease in ethanol (i.e. if acetaldehyde increased 10-15 μM, ethanol must concomitantly decrease 0.010-0.015 mM). Such a small ethanol decrease occurring amidst background blood ethanol values of 70-80 mM could not be readily detected. Therefore, small acetaldehyde increases would likely be easily detectible on the blood acetaldehyde curve, but not on the blood ethanol curve.

Blood pressure

Blood pressure depression in the experimental dogs might possibly be the result of blood acetaldehyde elevation which occurred coincidentally with the period of blood pressure depression. Although blood acetaldehyde levels associated with ethanol ingestion do not typically lead to blood pressure depression /9/, several studies in acetaldehyde challenged rats /10-12/ and in disulfiram or calcium cyanamide challenged humans /4,5/ have demonstrated central, hypotensive effects of acetaldehyde when elevated above levels associated with typical ethanol metabolism. Therefore, our finding of blood pressure depression associated with blood acetaldehyde elevation is consistent with the findings reported in the literature described above.

ACKNOWLEDGEMENTS

D.W. was supported by NIAAA 1 R29 AA08258 and the Georgia Agricultural Experiment Station.

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