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Metronidazole Influences the Development of Neural Tolerance to Ethanol

MARY L. A. GIKNIS AND IVAN DAMJANOV

Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine 230 North Broad Street, Philadelphia, PA 19102

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GIKNIS, M. L. A. AND I. DAMJANOV. *Metronidazole influences the development of neural tolerance to ethanol.* PHARMACOL BIOCHEM BEHAV **21**(2) 317–319, 1984.—The duration of the loss of the righting reflex (LoRR) was measured in Swiss Webster (SW) and DBA/2 mice following intraperitoneal injection of ethanol or metronidazole alone or together. A single injection of ethanol induced to short LoRR in SW mice and a long LoRR in DBA/2 mice. Metronidazole did not induce LoRR in either strain. When the mice were exposed daily to ethanol for five days, the duration of the LoRR was prolonged in SW mice and shortened in DBA/2 mice. This indicates the development of increased neural sensitivity to ethanol in SW mice and of neural tolerance to ethanol in DBA/2 mice. The response in SW mice to administration of ethanol and metronidazole together did not differ from their response to ethanol alone. However, the duration of the LoRR in DBA/2 mice injected repeatedly with the two drugs was longer than that observed with ethanol alone. Thus metronidazole appears to inhibit the development of neural tolerance to ethanol in DBA/2 mice but has no effect on ethanol induced LoRR in SW mice.

Ethanol Metronidazole Flagyl Sleep Genetics

METRONIDAZOLE (1-βhydroxyethyl)-2-methyl-5-nitroimidazole) is a potent trichomonacidal, amebicidal and bactericidal compound marketed worldwide [8]. In addition to antiparasitic and antibacterial effects, metronidazole inhibits certain mammalian liver enzymes which are involved in the catabolism of ethanol [4]. The ability of metronidazole to inhibit both alcohol dehydrogenase and aldehyde dehydrogenase has been cited as a possible explanation for the dizziness and nausea which occur in certain individuals following simultaneous ingestion of ethanol and metronidazole [3]. Since metronidazole frequently induces nausea when taken simultaneously with ethanol it has been used as an alternative to disulfiram in the treatment of chronic alcoholism [9]. However, nausea following simultaneous ingestion of metronidazole and ethanol does not occur in all alcoholics undergoing such treatment and therefore is not a universal phenomenon [9]. Although there are no clear explanations for the different responses observed among individual patients, the differential sensitivity is perhaps due to genetic differences similar to those which account for individual variations in response to ethanol [6].

In the present study, we have tried to determine whether the response to combined metronidazole-ethanol treatment is a strain-specific phenomenon. To this end we have used mice of two strains known to differ in their response to repeated injections of ethanol: Swiss Webster mice which develop increased neural sensitivity upon repeated exposure to ethanol and DBA/2 mice which develop neural tolerance under identical conditions [2]. We show that metronidazole does not potentiate the effects of ethanol in Swiss Webster mice. On the other hand, it affects the rate at which neural tolerance to ethanol develops in DBA/2 mice. Thus, we show the differential response to combined metronidazole-ethanol treatment could, at least in part, be based on genetic differences between exposed subjects.

METHOD

Swiss Webster (SW) mice were purchased from Perfection Breeders, Camden, New Jersey and DBA/2 mice from Jackson Laboratories, Bar Harbor, Maine. All experiments were performed on 8–10 week old virgin females, fed Purina Mouse Chow with access to tap water ad lib. The mice were housed, five mice per cage, in a standard animal facility and were maintained on a 12 hour light-dark cycle. All experiments were performed between 9 and 10 a.m. in the same animal room.

The mice of each strain were divided randomly into four groups of at least 10 animals each. Each group was injected intraperitoneally with one of the following solutions: a 25% solution of ethanol in saline (4 g/kg), a 25% solution of ethanol in saline followed immediately by a solution of metronidazole in saline (15 mg/kg), a solution of metronidazole in saline (15 mg/kg) or saline alone. The animals were injected every day for a total of five days. They were then allowed to recuperate for one week after which time they were again injected with the test substance(s). Following each injection, the loss of righting reflex (LoRR) of each animal was recorded and the mean duration of the loss of righting reflex after each treatment was calculated for each

[1]Requests for reprints should be addressed to M. L. A. Giknis, Stein Research Center, 920 Chancellor Street, Philadelphia, PA 19107.

group. After treatment, the animals either lost consciousness within 3–4 minutes or remained "awake." Following LoRR the animals were observed and placed on their backs. The duration of LoRR was defined as the interval from the moment the animal lost consciousness until it was able to right itself twice within 30 seconds. Upon the return of the righting reflex the animal was judged to be awake.

Blood ethanol concentrations were determined using the Sigma Chemical Company (St. Louis, MO) diagnostic kit No. 332-uv. Blood samples were taken from the retrobulbar plexus at 20, 60 and 120 minutes after the injection. The blood ethanol clearance rate was calculated by plotting the values for each animal as blood ethanol concentration versus time. The blood ethanol concentration at wakening, i.e., return of the righting reflex was extrapolated from the plotted data for each individual animal. The mean and standard error of the mean was then calculated for each experimental group. Data were analyzed using Student's t-test and linear regression analysis.

RESULTS

Following the first injection of ethanol the previously unexposed ("naive") SW mice experienced a short LoRR, whereas, the LoRR in naive DBA/2 mice was significantly longer (Fig. 1). Metronidazole injected alone did not induce LoRR. The duration of LoRR in naive animals injected for the first time simultaneously with ethanol and metronidazole did not differ from the duration of LoRR after exposure to ethanol alone in either strain of mice. Subsequent injections of ethanol prolonged LoRR of SW mice as did ethanol and metronidazole given together. The linear slope of the plotted data for the duration of LoRR following exposure to ethanol alone was 0.37 compared with a slope of 0.46 for the group exposed to ethanol and metronidazole together (no statistical difference $p>0.05$).

In contrast to SW mice, the DBA/2 mice injected with three or more consecutive doses of ethanol experienced a significantly shorted LoRR than the naive animals (Fig. 1). DBA/2 mice exposed on consecutive days to ethanol and metronidazole also experienced a shorter LoRR, but the shortening of the duration of LoRR was not as pronounced as in animals exposed to ethanol alone. The slope of the line determined for the duration of LoRR following exposure to ethanol alone was -0.40, compared with a slope of -0.10 for the mice exposed to ethanol and metronidazole together. This difference was statistically significant $t(6)=6.71$, $p<0.01$.

SW and DBA/2 mice treated with ethanol alone or ethanol and metronidazole together for five consecutive days were allowed to recover for seven days. Following the recovery period, the animals were again injected with either ethanol alone or ethanol and metronidazole together as per their original treatment regimen. The duration of LoRR in these mice was identical to the duration of LoRR in naive animals exposed for the first time, indicating that the animals had completely recovered from the previous treatment and had regained their original reactivity.

The rate of blood ethanol clearance and blood ethanol concentration at 20, 60 and 120 minutes did not differ among the mice of the two strains. The concomitant injection of metronidazole with ethanol did not effect the blood ethanol clearance rate in either the SW or DBA/2 mice (Table 1).

DISCUSSION

Various strains of mice differ in their response to ethanol

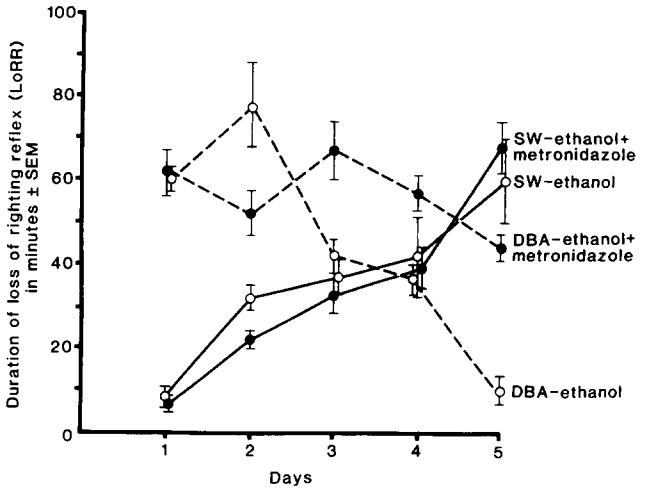

FIG. 1. Duration of loss of righting reflex in Swiss Webster (SW) and DBA/2 mice injected with ethanol or ethanol and metronidazole daily for five consecutive days. After the fifth injection the mice were allowed to recover for seven days and then injected again with the same drug combination. In SW mice there is no statistical difference $p>0.05$ in response to ethanol or ethanol combined with metronidazole. In DBA/2 mice the duration of loss of righting reflex was significantly longer in mice injected with ethanol and metronidazole than those injected with ethanol alone on day 3, 4, and 5 ($p<0.01$). The duration of loss of righting reflex on day 12 was the same as on day one of the experiment for each group.

[1, 5, 7]. In a previous report we have shown that DBA/2 mice develop neural tolerance following repeated exposure to ethanol, whereas, mice of other strains, namely C57BL/6, BALB/c, CD-1 and Swiss Webster (SW), develop increased neural sensitivity to ethanol after identical treatment [2]. Although we do not know the biochemical and physiological basis for the differential response to ethanol observed among various mouse strains, it seems possible that the duration of the loss of righting reflex following repeated exposure to ethanol has a genetic basis. We have thus proposed to use these strain-specific characteristics to study the neurobehavioural response to ethanol in the aforementioned mouse strains.

In the present study we have used two strains of mice which are known to differ in their response to repeated injections to ethanol hypothesizing that they should also differ in their response to combined ethanol-metronidazole treatment. In one strain, the SW mice, the combined drug treatment produced the same results as exposure to ethanol alone. However, in the other strain, the DBA/2 mice, metronidazole hindered the development of neural tolerance to ethanol. This modifying effect of metronidazole on the neurotrophic effects of ethanol in one but not the other mouse strain is an indication that the psycho-behavioral effects of metronidazole may be due to genetic differences between the mouse strains.

Our data provide no explanations for why metronidazole does not influence the development of increased neural sensitivity to ethanol in SW mice while slowing down the rate at which neural tolerance to ethanol develops in DBA/2 mice. There are, however, two possible explanations for these findings: either metronidazole inhibits ethanol catabolism

TABLE 1

BLOOD ETHANOL CLEARANCE RATES AND MAXIMUM BLOOD ETHANOL LEVELS MEASURED*

Strain of Mice	Treatment	Clearance rate (mg/ml/hr) ± S.E.M.	Maximum Blood Ethanol Level (mg/ml) ± S.E.M.* Measured
SW	Ethanol	0.89 ± 0.04	3.43 ± 0.2
	Ethanol/ Metronidazole	0.81 ± 0.16	3.51 ± 0.3
DBA/2	Ethanol	0.80 ± 0.09	3.56 ± 0.1
	Ethanol/ Metronidazole	0.77 ± 0.19	3.50 ± 0.2

No statistical difference in clearance rates of maximum blood ethanol level by Student's t-test. ($p>0.05$).

*Maximum blood ethanol level represents the blood ethanol level by 20 minutes after injection and was the highest blood ethanol level measured in these studies.

and retards the clearance of ethanol from the organism thus prolonging the exposure of nerve cells to high levels of ethanol in blood; or the drug in some way alters the responsiveness of nerve cells to ethanol. The first explanation seems unlikely since we show that metronidazole does not influence the clearance rate of ethanol from the blood. Therefore, our data suggest that metronidazole acts on the nerve cells by altering their reactivity to ethanol. It is not clear why metronidazole affects only the neural response of DBA/2 mice to ethanol and has no effect on that of the SW mice. The fact that the normal level of neural sensitivity was restored in both strains after a seven day recovery period suggests that the effects of metronidazole and ethanol were temporary and that no permanent neurologic damage was produced.

The present study was undertaken to provide some experimental data for the possible genetic basis of the so called "metronidazole effect," a peculiar occurrence of dizziness, nausea and mild neurological symptoms developing in some individuals who ingest ethanol and metronidazole at the same time [3,9]. Our findings show that metronidazole can indeed alter the neurotropic effects of ethanol and suggest that this murine "metronidazole effect" is influenced by the genetic differences between the mouse strains studied. The fact that the drug affected only mice of one strain and not the other supports the hypothesis that the "metronidazole effect" observed in man may also have a genetic basis and could explain why some individuals drinking alcoholic beverages while on metronidazole therapy develop dizziness and nausea while others are spared.

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