Pharmacology Biochemistry & Behavior, Vol. 40, pp. 513–515. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Tissue Glutathione Levels in Mice Treated With Delta-9-Tetrahydrocannabinol

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HUSAIN, S. AND K. M. AHMED. *Tissue glutathione levels in mice treated with delta-9-tetrahydrocannabinol.* PHARMACOL BIOCHEM BEHAV **40**(3) 513–515, 1991.—Glutathione (GSH) is widely distributed among living cells and is involved in many biological functions. It provides the sulfhydryl groups for conjugation of toxic metabolites of several xenobiotica. Acetaminophen (Tylenol) toxicity is a classical example of this property. For this purpose, we studied the effects of delta-9-tetrahydrocannabinol (THC) on tissue levels of GSH in the mice. Groups of male Swiss Webster mice weighing 25 ± 5 g were treated with 50 mg/kg, PO THC at 1300 h. Control mice were given equal volume of sesame oil (5 ml/kg, PO) which was the vehicle for THC. Ninety minutes following THC administration, mice were sacrificed, their plasma, brain, heart, liver, kidney and testis were collected. All tissues were homogenized in 5% TCA/EDTA solution and supernatant solutions of these homogenates were diluted. In these diluted samples, levels of GSH were determined by a modified spectrophotometric procedure and the GSH levels were expressed as micromoles of GSH/g tissue. In this study, THC caused no effects on GSH levels in brain, heart, testis and plasma. However, GSH levels in liver and kidney were decreased by 14% and 7% respectively. Although the decrease in kidney GSH levels were insignificant, these changes in liver and kidney could be indicative of a possible metabolic and/or dispositional interaction between THC and different commonly available drugs such as acetaminophen.

Tissue glutathione THC Marihuana Acetaminophen Tylenol Toxicity

GLUTATHIONE (GSH, gamma-glutamylcysteinylglycine) is the major nonprotein thiol widely distributed among living cells, particularly those of mammalian tissues (6). It plays an important role in a variety of metabolic process, some of which are critical for cell viability. Studies have shown that high concentrations of GSH are found in the liver of most species (17), and provides sulfhydryl groups for conjugation of toxic metabolites of several xenobiotica which decreases their toxicity (11,15).

Drugs of abuse such as marihuana and its major psychoactive constituent, delta-9-tetrahydrocannabinol (THC) have been studied extensively in laboratory animals as well as humans (3, 5, 13, 14). However, no studies are reported thus far which describe the effects of THC on GSH levels in different tissues of the body. This prompted us to determine the levels of GSH in mice with different treatments of THC. Similarly, large doses of the commonly used analgesic, acetaminophen (APAP, Tylenol) is known to cause hepatotoxicity by decreasing the levels of GSH in the body (1, 4, 8–11, 15). Due to the increased recreational use of marijuana in recent years, it is likely that it could be taken in close temporal proximity with the drugs sold over-the-counter for self-medication (e.g., APAP). This could lead to dispositional and/or metabolic interaction between these two drugs. For this purpose, in this investigation, we also studied the effects of a combination of THC and APAP on GSH levels in different tissues of mice.

METHOD

Male Swiss Webster mice (25–30 g) were obtained from Bio Lab Corp., Minneapolis, MN. They were maintained on standard laboratory chow and under alternate light/dark cycle of 12 h each. After one week of adaptation, animals were randomly divided into three groups. Animals in group A were given 50 mg/kg PO THC at 1300 h. Control mice were given equal volume of sesame oil (S.O.), 5 ml/kg, PO. These drugs were obtained from National Institute on Drug Abuse (NIDA), Rockville, MD. THC was obtained in ethanol. It was evaporated under nitrogen. The residue obtained after evaporation was dissolved in sesame oil (S.O.). Ninety minutes following drug administration, mice were sacrificed by decapitation. Their blood, brain, heart, liver, kidney and testes were obtained. Blood was collected in a heparinized tube and centrifuged. Animals in group B were administered 350 mg/kg, IP APAP dissolved in 0.9% basic saline, pH 11.3. Control mice were given 10 ml/kg, IP saline. Basic saline was used because APAP has better solubility in it and also it has no effect on hepatic GSH levels (12). APAP was administered at 1200 h. Two h following its administration, animals were sacrificed and their plasma and other tissues were obtained as before. Animals in group C were given APAP (350 mg/kg, IP) at 1200 h. Control mice received equal volume of saline. At 1230 h, 30 min after APAP administration, all mice including controls, were treated with THC (50 mg/kg, PO). This treatment dose of THC and that of APAP were selected because of personal observations and information from other laboratories which indicate that at this dose, both drugs cause significant effects in different biological systems (8–10, 13). Mice were sacrificed at 1400 h and their plasma and tissues were obtained as earlier. Tissues from all groups were washed in saline, weighed and kept on ice until homogenized in 5%

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TABLE 1

PLASMA AND TISSUE GLUTATHIONE LEVELS IN MICE FOLLOWING Δ^9-TETRAHYDROCANNABINOL TREATMENT*

Tissue		Glutathione Levels μmol/g Tissue†	Percent Change From Control	Significance‡
Plasma	Control	0.24 ± 0.02 (9)		
	Test	0.25 ± 0.01 (9)	+ 4.0	N.S.
Brain	Control	2.13 ± 0.06 (10)		
	Test	2.09 ± 0.08 (10)	− 2.0	N.S.
Heart	Control	2.66 ± 0.05 (10)		
	Test	2.62 ± 0.12 (10)	− 2.0	N.S.
Liver	Control	7.60 ± 0.34 (10)		
	Test	6.55 ± 0.29 (10)	−14.0	$p<0.03$
Kidney	Control	6.67 ± 0.27 (9)		
	Test	6.23 ± 0.26 (9)	− 7.0	N.S.
Testis	Control	4.01 ± 0.07 (10)		
	Test	4.08 ± 0.23 (10)	+ 2.0	N.S.

*Control and test mice were treated respectively with 5 ml/kg, PO sesame oil and 50 mg/kg, PO THC. Animals were sacrificed 90 min postinjection and tissues analyzed to determine glutathione levels as described in the Method section.

†Mean glutathione levels ± S.E.M. with number of animals in parentheses.

‡Significance determined using Student's t-test, $p<0.05$.

TCA/EDTA solution to obtain a 20% w/v homogenate. These homogenates were centrifuged for 10 min at 5000 rpm to obtain a protein-free, clear supernatant solution. Aliquots of these supernatants were diluted (liver, kidney 5×, other tissues 2×) with 5% TCA/EDTA and their levels of nonprotein sulfhydryl groups (NPSH) were determined by a modified spectophotometric procedure (16). It is generally accepted that NPSH is approximately equivalent to GSH in most mammalian tissues including liver and kidney (2,6). Therefore, the NPSH levels were expressed as micromoles of GSH/g tissue. These results are presented as mean value ± SEM. Significance was determined by Student's t-test and the acceptable level of significance was set at $p<0.05$.

TABLE 2

PLASMA AND TISSUE GLUTATHIONE LEVELS IN MICE FOLLOWING ACETAMINOPHEN TREATMENT*

Tissue		Glutathione Levels μmol/g Tissue†	Percent Change From Control	Significance‡
Plasma	Control	0.28 ± 0.04 (4)		
	Test	0.27 ± 0.02 (4)	− 4.0	N.S.
Brain	Control	1.66 ± 0.09 (10)		
	Test	1.57 ± 0.12 (5)	− 5.0	N.S.
Heart	Control	2.16 ± 0.17 (8)		
	Test	2.40 ± 0.21 (5)	+11.0	N.S.
Liver	Control	6.95 ± 0.29 (9)		
	Test	3.44 ± 0.33 (5)	−51.0	$p<0.0001$
Kidney	Control	4.79 ± 0.33 (10)		
	Test	4.61 ± 0.28 (5)	− 4.0	N.S.
Testis	Control	3.28 ± 0.15 (9)		
	Test	3.09 ± 0.21 (5)	− 6.0	N.S.

*Control and test mice were treated respectively with 10 ml/kg, IP saline and 350 mg/kg, IP APAP. Animals were sacrificed 2 h postinjection and tissues analyzed to determine glutathione levels as described in the Method section.

†Mean glutathione levels ± S.E.M. with number of animals in parentheses.

‡Significance determined using Student's t-test, $p<0.05$.

TABLE 3

PLASMA AND TISSUE GLUTATHIONE LEVELS IN MICE FOLLOWING COMBINED TREATMENT WITH Δ^9-TETRAHYDROCANNABINOL AND ACETAMINOPHEN*

Tissue		Glutathione Levels μmol/g Tissue†	Percent Change From Control	Significance‡
Plasma	Control	0.31 ± 0.01 (5)		
	Test	0.29 ± 0.03 (6)	− 6.0	N.S.
Brain	Control	2.10 ± 0.06 (11)		
	Test	2.01 ± 0.06 (11)	− 4.0	N.S.
Heart	Control	2.65 ± 0.10 (11)		
	Test	2.60 ± 0.11 (11)	− 2.0	N.S.
Liver	Control	7.30 ± 0.35 (11)		
	Test	3.65 ± 0.12 (11)	−50.0	$p<0.0001$
Kidney	Control	6.11 ± 0.23 (11)		
	Test	4.71 ± 0.18 (11)	−23.0	$p<0.0002$
Testis	Control	3.53 ± 0.13 (11)		
	Test	3.51 ± 0.11 (11)	− 1.0	N.S.

*Control mice were treated with 10 ml/kg, IP saline and 30 min after with 50 mg/kg, PO THC. Test mice were treated with 350 mg/kg, IP APAP and 30 min after with 50 mg/kg, PO THC. Animals were sacrificed 2 h after the first injection and tissues analyzed to determine glutathione levels as described in the Method section.

†Mean glutathione levels ± S.E.M. with number of animals in parentheses.

‡Significance determined using Student's t-test, $p<0.05$.

RESULTS AND DISCUSSION

In this study, levels of GSH were a parameter considered to evaluate drug effect on various organs in mice. All experiments were conducted around 1400 h. This was done because nocturnal feeding in mice causes large variability in GSH levels. This variability is minimized if the experiments are run at time points farther removed from nocturnal feeding (12). The mice which were treated with THC in group A showed the highest level of GSH in the liver followed by kidney, testis, heart and brain. However, in plasma, the level of GSH was the lowest (Table 1). These results indicate that as compared to controls, THC caused no effect on GSH levels in brain, heart, testis and plasma. However, GSH levels in liver and kidney were decreased by 14% and 7%, respectively with the decrease being significant in the liver as compared to controls (7.60 ± 0.34 vs. 6.55 ± 0.29 μmol GSH/g tissue). Treatment of mice with APAP in group B showed a 51% significant decrease in hepatic GSH levels (6.95 ± 0.29 vs. 3.44 ± 0.33 μmol GSH/g tissue). There was no significant difference in the levels of GSH in other tissues (Table 2). This is in agreement with earlier reports from other laboratories which indicate that both in mice and rats, kidney shows neither necrosis nor any other specific changes after various doses of APAP (8). In our present experiments, we also observed no changes in the level of GSH in kidney of mice treated with APAP. As opposed to this, there are reports in literature which establish that glutathione has an important role in the kidney (7). On the basis of these reports and our laboratory findings, we were further interested to study the levels of glutathione in mice following a combined treatment with THC (50 mg/kg, PO) and APAP (350 mg/kg, IP). The results of this

study indicated that there was no combined effect of this concurrent treatment on the levels of glutathione in the liver and other tissues except kidney (Table 3 vs. 1 and 2). In kidney, there was a significant combined effect (23%) of this concurrent treatment as compared to the effects of THC or APAP alone (Table 3 vs. 1 and 2). However, from the present findings, it is evident that the concurrent treatment of mice with THC and APAP failed to show significant difference in hepatic glutathione levels. It is, therefore, speculated that no metabolic interaction occurs when these two drugs are taken together. However, in kidney, the significant decrease in glutathione levels following concurrent treatment suggests the possibility of a dispositional interaction between THC and APAP when taken together.

ACKNOWLEDGEMENTS

This research was supported by National Institute on Drug Abuse (NIDA) Grant DA05215. We wish to thank NIDA for providing THC for this study. The assistance of Ms. Debbie Kroese in typing this manuscript is also appreciated.

REFERENCES

1. Black, M. Acetaminophen hepatotoxicity. Annu. Rev. Med. 35: 577–593; 1984.
2. Boyd, S. C.; Sesame, H. A.; Boyd, M. R. High concentrations of glutathione in glandular stomach. Possible implications of carcinogenesis. Science 205:1010–1012; 1979.
3. Hafen, B. Q.; Frandsen, K. J. Marijuana facts, figures and information for the 1980s. Minneapolis, MN: Hazelton Foundation Inc.; 1981.
4. Hinson, J. A.; Phol, L. R.; Monks, T. J.; Gillette, J. R. Mini Review: Acetaminophen induced hepatotoxicity. Life Sci. 29:107–116; 1981.
5. Husain, S. Marijuana abuse: Its pharmacology and effects on testicular function. In: Redda, K.; Walker, C.; Barnett, G., eds. Cocaine, marijuana and designer drugs: Chemistry, pharmacology and behavior. Boca Raton, FL: CRC Press; 1989:127–143.
6. Jocelyn, P. C. Biochemistry of SH group. New York: Academic Press; 1972.
7. Meister, A. On the enzymology of amino acid transport. Science 180:33–39; 1973.
8. Mitchell, J. R.; Jollow, D. J.; Potter, W. Z.; Davis, D. C.; Gillette, J. R.; Brodie, B. B. Acetaminophen induced hepatic necrosis. 1 Role of drug metabolism. J. Pharmacol. Exp. Ther. 187:185–194; 1973.
9. Mitchell, J. R.; Jollow, D. J.; Potter, W. Z.; Gillette, J. R.; Brodie, B. B. Acetaminophen induced hepatic necrosis. IV Protective role of glutathione. J. Pharmacol. Exp. Ther. 187:211–217; 1973.
10. Mitchell, J. R.; Thorgeirsson, S. S.; Potter, W. Z.; Jollow, D. J.; Keiser, H. Acetaminophen induced hepatic injury: Protective role of glutathione in man and rationale for therapy. J. Clin. Pharmacol. Ther. 16:676–684; 1974.
11. Park, Y.; Smith, A. B.; Combs, A. B.; Kehrer, J. P. Prevention of acetaminophen induced hepatotoxicity of dimethyl sulfoxide. Toxicology 52:165–175; 1988.
12. Peterson, F. J.; Knodell, R. G.; Landemann, N. J.; Steele, N. M. Prevention of acetaminophen and cocaine hepatotoxicity in mice by cimetidine treatment. Gastroenterology 85:122–129; 1983.
13. Pryor, G. T.; Husain, S.; Mitoma, C. Acute and subacute interactions between Δ^9-tetrahydrocannabinol and other drugs in the rats. Ann. NY Acad. Sci. 281:171–189; 1976.
14. Relman, A. S. Marijuana and Health. Washington, DC: Institute of Medicine, Division of Health Science Policy, National Academy Press; 1982.
15. Saville, J. G.; Davidson, C. D.; D'Adrea, G. H.; Born, G. K.; Hamrick, M. E. Inhibition of acetaminophen hepatotoxicity by chlorpromazine in fed and fasted mice. Biochem. Pharmacol. 37: 2467–2471; 1988.
16. Sedlak, J.; Lindsay, R. H. Estimation of total, protein bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25:192–205; 1968.
17. Wills, E. D. The role of glutathione in drug metabolism and the protection of the liver against toxic metabolites. In: Gorrold, J. W., ed. Testing for toxicity. London: Taylor and Francis, Ltd.; 1981: 95–109.