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The Effect of Halothane on the Enzymatic Activity of Mouse Liver and Erythrocyte Glucose-6-Phosphate Dehydrogenase

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Abstract: The effects of repeated halothane anaesthesia on 6-10 week old male, *Mus musculus* albino mice weighing 29-32 g were studied. Halothane, a widely used anaesthetic, is known to be metabolised in the liver to toxic intermediates through cytochrome P4502E1 (CYP2E1). During the first week of repeated dosage, glucose-6-phosphate dehydrogenase (G6PDH) activity decreased in both erythrocytes and liver cells, and during this time, livers increased significantly in weight and suffered histopathological damage, the damage increasing with the dosage of halothane, in the range 0.25-1.0 ml twice daily. When repeated anaesthesia was continued for a second week, G6PDH activity increased significantly and histopathological damage to the liver showed no further increase. Human and mouse G6PDH show homology, and further work to examine the parameters controlling the eventual increase of enzyme activity in response to insult by halothane and its metabolites would be worthwhile.

Key Words: Halothane, G6PDH, RBC, liver.

Introduction

Halothane, 2-bromo-2 chloro-1-1-trifluoroethane, has been used widely as an inhaled anaesthetic since 1957 (1). It is well established that halothane is metabolised in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by monooxygenases through the cytochrome P4502E1 system (CYP2E1) (2,3). Thus, halothane anaesthesia causes hepatocellular necrosis, destruction of the lipid-protein interactions in human erythrocyte membranes, decrease in the activities of membrane enzymes and alteration of cerebral glucose-6-phosphate dehydrogenase (E.C.1.1.1.49, G6PDH) activities (4). G6PDH, a key regulatory enzyme of the pentose phosphate pathway, furnishes the reduced nicotinamide adenine dinucleotide phosphate (NADPH) used by the CYP2E1 system. Free radicals generated during oxidative stress are quenched by such antioxidants as glutathione (GSH) and antioxidant enzymes, the final electron acceptor being NADPH. Thus, G6PDH activity can be used as a biomarker to probe the effects of detoxification on anaesthesia.

In order to gain further insight, we explore the effects of repeated anaesthesia by halothane on mice, especially on their livers. The investigation emphasizes measurements of G6PDH activity since human and mouse G6PDH enzymes show homology (5).

Materials and Methods

Forty-five male, non-inbred, albino *Mus musculus* mice, 6-10 weeks old, weighing between 29 and 33 g, were obtained from the Medical Sciences Experimental Research Center of Cukurova University. The animals were divided into three groups: a control group of fifteen apparently normal mice, and groups I and II, consisting of fifteen mice each, which were repeatedly anaesthetised with halothane for 1 or 2 weeks, respectively. According to the volume of halothane inhaled on each occasion, groups I and II were subdivided into three subgroups: a, b and c, with 0.25 ml, 0.50 ml and 1.00 ml, respectively. Inhalation persisted for 30 minutes at 0800 and 1600 hours, daily. Throughout the experiment all mice were given tap water and a standard laboratory diet. The room temperature was maintained at 22-24°C and the illumination (12 hour light\dark cycle) was constant. Blood was extracted from the tail vein and, after anaesthesia, from the heart. The mice were weighed and sacrificed. The livers were quickly removed, weighed and homogenised with three volumes of ice-cold 0.25 M

sucrose. The activity of G6PDH enzyme was measured in the supernatant obtained from centrifugation at 105,000 g.

Enzyme activity was determined at 37°C both in the homogenate and in the hemolysate according to the procedure of Beutler (6). Protein concentrations were measured using bovine serum albumin as the standard (7) and hemoglobin was determined by the cyanomethemoglobin method (6).

Liver tissues were fixed in 10% formaldehyde and embedded in paraffin. Five micrometer sections were obtained, stained with Harris hematoxylene-eosin and examined under a light microscope (8).

All chemicals used in enzyme assays were analytical grade and were from the Sigma Chemical Company (St. Louis, MO, USA).

Results

Superficially, repeated anaesthesia, even for two weeks, had no obvious effect on either the activity or the health of the mice. Microscopic examination (Figure) showed specific morphological damage to have been caused to liver tissues during the first week of exposure to halothane, the extent of the damage increasing with the dosage of the anaesthetic. Eosinophilic leucocytes and mononuclear inflammatory cell infiltration of the portal tractus were observed in the livers of three mice (one in subgroup Ia, two in subgroup Ib). Nineteen livers suffered cloudy degenerations, congestions, micro and macro vesicular fatty degeneration, spotty necrosis and centrilobular necrosis of the parenchymal cells. Liver damage persisted but did not increase further in the second week of repeated anaesthesia.

Whereas anaesthesia had no significant effect on the total body weight ($P>0.05$) of the mice, it did increase the weight of the livers significantly, except in subgroup IIc (Table 1). The distinction between biochemical and physiological response to halothane in the first and second weeks of repeated anaesthesia is shown by the measured activities of G6PDH. Table 2 shows the G6PDH activity of erythrocytes to have decreased significantly during the first week of repeated anaesthesia but to have returned to the 'control' value by the second week and increased significantly in the highest dosage. The same phenomenon for G6PDH in the mouse liver is shown in Table 2. Repeated anaesthesia with dosages of 0.5 or 1.0 ml of halothane for 1 week reduced the enzyme activity by a factor of two or three, but during the second week of repeated anaesthesia G6PDH activity returned to at least its control value.

Discussion

Halothane is metabolised to hepatotoxic intermediates by an oxidative CYP2E1 dependent pathway (2,3). The biotransformation occurs through the generation of free radicals, as has been shown by spin-trapping experiments and electron spin resonance (9). In general, livers increased in weight, as shown in Table 1. However, the highest dosage and longer duration of exposure to anaesthesia seemed to inhibit the increase. These results are similar to the effects of ozone (10), which caused only a slight decrease in the body weights of exposed mice, but a significant increase in the weights of the lungs. G6PDH activity decreased significantly both in the erythrocyte and in the liver during the first week of exposure to halothane, the magnitude of these changes in activity paralleling the dose of the anaesthetic. G6PDH seems to be utilized in the defense mechanism against the free radical generated in the metabolism of halothane. However, when exposure to halothane was continued for a second week, enzyme activity returned to its normal

Table 1. Liver weights in control and anaesthetised mice.

Groups	n	Exposure (week)	Dose (ml 2x daily)	Liver weights (g) X±SD	range
Control	15	0	0	1.37±0.10	1.02-1.75
*Ia	5	1	0.25	2.00±0.32	1.56-2.36
*Ib	5	1	0.50	2.17±0.47	1.67-2.71
*Ic	5	1	1.00	1.98±0.31	1.70-2.42
*IIa	5	2	0.25	2.79±0.66	2.23-3.93
*IIb	5	2	0.50	2.13±0.54	1.53-2.98
IIc	5	2	1.00	1.54±0.26	1.31-1.99

* Livers of anaesthetised mice weighed significantly more ($p<0.05$) than livers of controls except subgroup IIc (Mann-Whitney-U test).

Table 2. RBC and liver G6PDH activities of control and anaesthetised mice.

Groups	RBC G6PDH activities	Liver G6PDH activities	
	U/g Hb (X±SD)	U/mg protein (X±SD)	U/g liver (X±SD)
Control	32.4±7.7	0.036±0.017	1.756±0.814
Halothane 1 week			
Ia (0.25 ml)	*22.6±9.5	0.030±0.016	1.621±1.006
Ib (0.50 ml)	*25.3±8.7	*0.017±0.009	*0.546±0.241
Ic (1.00 ml)	*20.0±11.6	*0.011±0.004	*0.533±0.194
Halothane 2 weeks			
IIa (0.25 ml)	35.4±3.8	+0.052±0.037	+3.239±0.756
IIb (0.50 ml)	39.8±3.4	0.039±0.011	+2.498±1.193
IIc (1.00 ml)	+42.4±3.2	0.028±0.008	1.840±0.832

* RBC and liver G6PDH activities decreased significantly in the first week of anaesthesia compared to control group ($p<0.05$) (Mann-Whitney-U test).

\+ RBC and liver G6PDH activities increased significantly in the second week of anaesthesia compared to control group ($p<0.05$) (Mann-Whitney-U test).

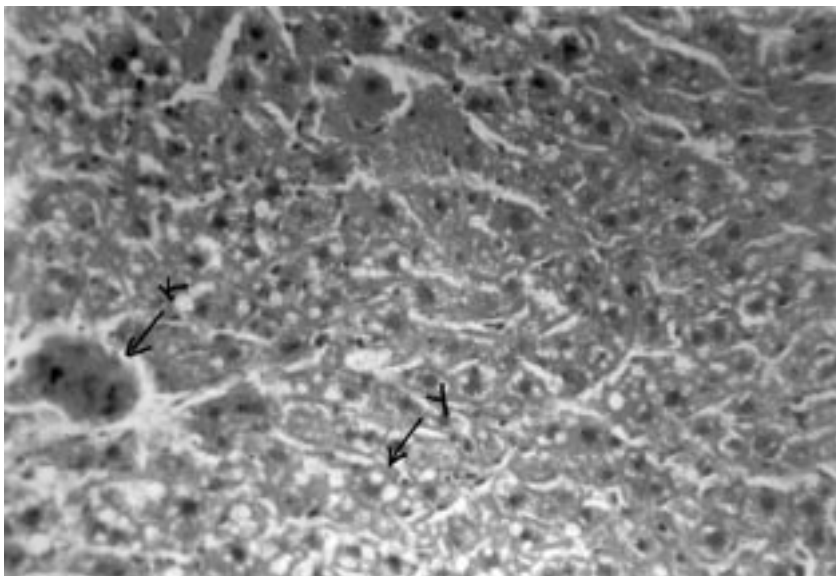

Figure 1. Liver tissue taken from anaesthetised group (IIC) of *Mus musculus* albino mouse (Hematoxylene-Eosin. x200). K: congestion, Y: micro and macro vesicular fatty degeneration.

value. Indeed, during the second week of repeated anaesthesia, G6PDH activity in the liver expressed as units per g of liver appeared to exceed the control value, suggesting that the synthesis of enzymes in the liver may have been increased. This is entirely consistent with the previous works of Caneghem (11) and Ankrah (12), who showed that phenylhydrazine and aflatoxin B1 caused temporary induction of G6PDH activity in mouse liver.

These results illustrate the 'fine' and 'coarse control' of G6PDH. G6PDH is a regulatory enzyme showing end-product inhibition and deinhibition by the phospho/dephospho mechanism, the NADPH/NADP ratio, the GSSG/GSH ratio and protein attachment/deattachment to the cellular membrane (13). Thus in the erythrocyte any of these mechanisms would control the free radical assault. However, in the liver, in order to adapt to the life-

threatening attack, the 'coarse control', which responds to such external stimuli as hormones, growth factors, nutrients and oxidant stress, promotes the increased synthesis of enzymes (13,14).

Damage to liver cells occurred during the first week of repeated anaesthesia whilst G6PDH activity was lowered. During the second week, though damage persisted, induction of G6PDH was observed. Further work to examine the parameters controlling the delay in induction would be worthwhile.

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