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Increased toxicity of the antitumor drug cyclophosphamide in mice in the presence of the volatile anesthetic agent halothane

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Summary. Exposure of mice to 0.5% halothane in air, which is close to a maintenance concentration in man, after an IP dose of cyclophosphamide produced an increase in the lethality of cyclophosphamide. The LD_{50} (30 day) for cyclophosphamide without halothane was 251 mg/kg; with 2 h subsequent exposure to halothane it was 152 mg/kg; and with 20 h subsequent exposure to halothane it was 158 mg/kg. The median survival time of mice receiving cyclophosphamide at doses between 137 and 240 mg/kg was more than 30 days in the absence of halothane, 12 days with 2 h halothane, and 10.5 days with 20 h halothane exposure. Survival of mice was decreased irrespective of whether 2 h halothane exposure preceded or followed cyclophosphamide administration. Separation of cyclophosphamide administration and preexposure to halothane by breathing air for 1 h abolished the decrease in survival. Halothane exposure for 2 h after cyclophosphamide had no effect on the antitumor activity of cyclophosphamide. Total-body clearance of cyclophosphamide in mice exposed to halothane was 60 ml/min/kg, as against 188 ml/min/kg in nonexposed mice. No change was produced by halothane in the area under the plasma concentration-time curve over 2 h for 4-hydroxycyclophosphamide following cyclophosphamide administration. The reason for the increased lethality of cyclophosphamide in the presence of halothane could not be determined. There was no increase in leukopenia caused by cyclophosphamide and no increase in bladder toxicity, in liver toxicity, in renal toxicity, or in the penetration of cyclophosphamide into the brain. The study, together with reports of increased toxicity in patients receiving cancer chemotherapy in close proximity to general anesthesia, should alert physicians and others to the possibility of an interaction between volatile anesthetic agents and chemotherapeutic drugs.

Introduction

Anticancer drugs generally have a small therapeutic index and are administered to patients at doses close to a toxic dose. A small increase in biological activity resulting from an interaction between an anticancer drug and another pharmacological agent could easily result in serious toxicity to the patient. The consequences of interactions between one anticancer drug and another anticancer drug leading to increased toxicity are well known [4]. Less is known about the effects of interactions between anticancer drugs and other pharmacological agents [9]. Drug interactions may occur in patients receiving anticancer drugs and anesthetic agents in close proximity [5]. There are reports of severe toxicity, and even death, of patients ascribed to an interaction of volatile anesthetic agents with anticancer drugs [27]. Bruce [2] reported an increase in the lethality of cyclophosphamide in mice and rabbits exposed for long periods to the volatile anesthetic agent halothane. The present study was conducted to determine whether a toxic interaction occurred in mice between cyclophosphamide and halothane given for fairly short periods such as might be used during surgical procedures in humans and to investigate possible mechanisms for the increased lethality. A preliminary report of this work has been presented elsewhere [29].

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Materials and methods

Male BDF_1 mice (Sprague-Dawley, Madison, Wis) weighing 18–24 g were used throughout the study. Cyclophosphamide (2H-1,3,2-monohydrate oxazaphosphorine, Sigma Chemical Co., St. Louis, Mo) was injected IP dissolved in all cases in 0.1 ml 0.9% NaCl. Control animals received 0.1 ml 0.9% NaCl only. Halothane (Fluothane, Ayerst Laboratories, New York, NY) was delivered by means of a Fluotec dispenser (Cyprane Ltd., UK) at a concentration of 0.5% halothane in air. Mice were housed in a large plexiglass box with a fan to circulate the air/halothane mixture, and were allowed free access to water but not to food for the period of exposure. The flow of 0.5% halothane in air to the box was 4 l/min. The concentration of halothane used, 0.5%, is approximately half the minimum alveolar concentration (MAC) [10], and although the mice were lethargic they were not completely anesthetized and could move around the box. For lethality studies groups of 12 mice received cyclophosphamide at doses of 95–600 mg/kg and were then exposed to 0.5% halothane for 2 h or 20 h. Deaths were recorded daily for 30 days. In other studies groups of 6 mice were preexposed to halothane for 2 h, after which cyclophosphamide was administered at a dose of 300 mg/kg and the mice were exposed to 0.5% halothane for a further 2 h or 20 h.

Antitumor activity against murine leukemia P388 was determined according to National Cancer Institute proto-

cols [12]. Mice were inoculated IP with 10^6 leukemia P388 cells. At 24 h later groups of 6 mice were exposed to 0.5% halothane for 2 h, and then received cyclophosphamide IP at 90 mg/kg, which is the optimum dose for antitumor activity against leukemia P388 and the approximate LD_{10} in BDF_1 mice [15], or at 30 mg/kg, and were exposed to 0.5% halothane for a further 18 h. Other mice were inoculated intracerebrally at the frontal-parietal junction, with 10^6 leukemia P388 cells. At 24 h later groups of 6 mice were given cyclophosphamide IP at 30 or 90 mg/kg and exposed to 0.5% halothane for 2 h or 20 h. Deaths were recorded daily for 30 days.

For pharmacokinetic studies groups of 6 mice received cyclophosphamide at a dose of 65 mg/kg IP. The mice were exposed to 0.5% halothane for 5 min before receiving cyclophosphamide, and afterwards for up to 120 min. Mice were killed by exsanguination achieved by orbital sinus puncture [22]. Blood was collected into chilled heparinized tubes and plasma separated rapidly with a microcentrifuge (Microfuge-B, Beckman Instruments, Fullerton, Calif). A sample of plasma was taken immediately for assay of 4-hydroxycyclophosphamide, and another sample was stored at $-70°$ until assay of cyclophosphamide. Cyclophosphamide was measured by a modification of the gas chromatographic procedure of Juma et al. [18] with a OV-101 capillary column. Hydroxycyclophosphamide was measured by a fluorometric procedure described by Volcker and Hohorst [30]. For studies of the penetration of cyclophosphamide and its metabolites into the brain, groups of 6 non-tumor-bearing mice received [ring-4-^{14}C]-cyclophosphamide (New England Nuclear, Boston, Mass), 65 mg/kg, 4 μCi/mouse IP. After 20 min the mice were killed by exsanguination achieved by orbital sinus puncture, and the brains were removed and homogenized in 5 ml ice-cold water. Radiolabeled cyclophosphamide and polar cyclophosphamide metabolites in the brain and plasma were measured by the procedure of Mellet et al. [20]. To a 1-ml sample of brain homogenate or plasma was added 1 mg unlabeled cyclophosphamide, 2 g NaCl, and 1 ml 1 *M* phosphate buffer, pH 7.0. Cyclophosphamide was extracted twice with 10 ml ethylene dichloride containing 10% methanol, and the extracts were combined. The organic solvent was evaporated under N_2 and the residue dissolved in 0.8 ml *n*-amyl alcohol prior to liquid scintillation counting. Whole brain homogenate or plasma was digested with Biosolve (Beckman Instruments, Fullerton, Calif) prior to liquid scintillation counting. Polar metabolites, expressed as cyclophosphamide equivalents, were calculated as the difference between the total radioactivity and the organic solvent-extractable radioactivity. Cyclophosphamide in plasma and brain homogenate was also determined by the gas chromatographic procedure described previously.

The effect of halothane on the leukopenic effect of cyclophosphamide was assessed by measuring peripheral white blood cells. Groups of four mice received cyclophosphamide, 100 mg/kg IP, and were then exposed to 0.5% halothane for 2 h. Samples of blood, 0.1 ml, were collected by orbital sinus puncture into heparinized tubes on days 0 (before treatment), 1, 3, 5, 7, 9, and 12. Total white blood cell count was determined with a Coulter counter (Model ZBI, Coulter Electronics Inc., Hialeah, Fla).

The bladder toxicity of cyclophosphamide was measured by the increase in bladder weight [14] of groups of 6 mice 2 days after administration of intraperitoneal cyclophosphamide at doses of 100 or 350 mg/kg, with and without subsequent exposure to 0.5% halothane for 2 h. Liver toxicity was measured by an increase in serum total bilirubin and serum glutamate-pyruvate transaminase, and renal toxicity by an increase in blood urea nitrogen, in groups of 6 mice 3 days after IP administration of cyclophosphamide, 350 mg/kg, with and without subsequent exposure to 0.5% halothane for 2 h. Serum total bilirubin and blood urea nitrogen were measured using Sigma diagnostic test kits (Sigma Chemical, St. Louis, Mo) and serum glutamate pyruvate transaminase with a diagnostic test kit from Beckman (Beckman Instruments, Fullerton Calif).

Pharmacokinetic analysis of cyclophosphamide plasma concentration data was conducted by using the NONLIN least-squares regression analysis computer program [21] and a weighting factor of $1/y^2$. Because insufficient data were available for accurate definition of the absorption phase of cyclophosphamide disposition following IP administration an infusion time of 5 min was assumed in the calculation of total-body clearance.

Statistical analysis of mouse lethality data exploited Probit analysis for the estimation of lethality curves and the coefficient of slope of the Probit equation was used for comparison of the lethality curves. Survival data from studies of antitumor activity were compared according to the Mann-Whitney test, with correction for ties. Other data were compared with the aid of Student's *t*-test [26].

Results

Lethality

An initial study on the effect of subsequent exposure to 0.5% halothane in air on the lethality of cyclophosphamide, with six mice per dose level of cyclophosphamide,

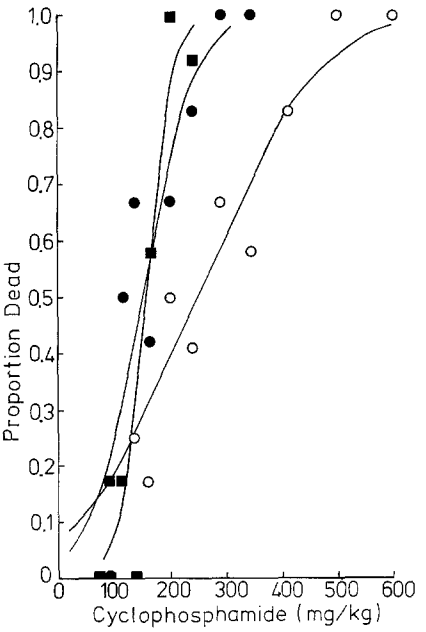

Fig. 1. Effect of halothane on lethality of cyclophosphamide. Groups of 12 mice received cyclophosphamide IP at each dose level and were exposed to (○) air; (●) 0.5% halothane in air for 2 h; or (■) 0.5% halothane in air for 20 h. *Continuous lines* are the computer-derived lethality curves over 30 days

showed a decrease in the LD_{50} of cyclophosphamide from 261 mg/kg in control mice to 185 mg/kg in mice exposed to halothane for 2 h and 158 mg/kg in mice exposed to halothane for 20 h. Exposure to this concentration of halothane alone for up to 20 h was not lethal to BDF_1 mice. The study was repeated with 12 mice per dose of cyclophosphamide, and the results of this study are shown in Fig. 1 and Table 1. Halothane exposure increased the slope of the cyclophosphamide lethality curve. The slope of the Probit equation of the cyclophosphamide lethality curve was increased by a factor of 2.21 ($P<0.05$) by 2-h halothane exposure and by a factor of 4.05 ($P<0.01$) by 20-h halothane exposure, compared with the slope of the Probit equation in the absence of halothane. Halothane exposure for 2 h or 20 h decreased the LD_{50} and LD_{90} for cyclophosphamide to about the same extent (Table 1). The median survival time of mice receiving cyclophosphamide at doses between 137 and 240 mg/kg was more than 30 days in the absence of halothane, 12 days ($P<0.01$) with 2-h halothane exposure, and 10.5 days ($P<0.01$) with 20-h halothane exposure, where the P-values are for the log-rank test of the halothane-treated groups versus the non-halothane-treated group. The median survival time of mice that died after receiving cyclophosphamide in the same dose range was 9 days in the absence of halothane, 11 days with 2-h halothane exposure, and 9 days with 20-h halothane exposure.

In other studies the sequence of halothane exposure and administration of a single dose of cyclophosphamide, 300 mg/kg, was varied (Table 2). It should be noted that there was significant variability in the response of different groups of mice to cyclophosphamide. In this study a cyclophosphamide dose of 300 mg/kg, which is above the LD_{50} for control mice in the previous studies, produced only one death by day 30 in the six control mice. Previous exposure to 0.5% halothane for 2 h before cyclophosphamide

Table 1. Effect of halothane on cyclophosphamide lethality

	Control m/kg	2 h Halothane mg/kg	20 h Halothane mg/kg
LD_{50}	250.6 (196.1, 299.2)	151.7 (77.6, 199.2)	157.9 (138.8, 176.0)
LD_{90}	462.6 (389.4, 637.0)	247.8 (200.0, 479.2)	210.6 (189.9, 250.2)

Values (together with their 95% confidence intervals) are calculated from data shown in Fig. 1 by Probit analysis and are based on deaths over a 30-day period

Table 2. Survival of mice given cyclophosphamide and halothane

	Median survival (days)	30-Day survivors
Control	> 30	6/6
Cyclo	> 30	5/6
Hal (2 h)	> 30	6/6
Hal (20 h)	> 30	6/6
Hal (2 h) → Cyclo	8	3/6
Hal (2 h) → Air (1 h) → Cyclo	> 30	5/6
Hal (2 h) → Cyclo → Hal (2 h)	3	3/6
Hal (2 h) → Cyclo → Hal (20 h)	4	0/6

Groups of six mice received cyclophosphamide (Cyclo) 300 mg/kg IP and/or were exposed to 0.5% halothane (Hal) in air for various times in the sequence shown. Deaths were recorded each day for 30 days

Table 3. Effect of halothane on antitumor activity of cyclophosphamide

	Without halothane		With halothane	
	Median survival (days)	30-day survivors	Median survival (days)	30-day survivors
No drug treatment	10	0/4	10	0/4
Cyclophosphamide				
30 mg/kg	14	0/4	14	0/4
90 mg/kg	21	0/4	20	0/4

Groups of four mice received tumor transplants IP, with 10^6 leukemia P-388 cells and 24 h later, cyclophosphamide IP at the doses shown. This was preceded by a 2-h exposure to 0.5% halothane and an 18-h exposure to 0.5% halothane after cyclophosphamide. Values are Kaplan–Meier median survival times

resulted in a median survival time of the mice of 8 days, as against more than 30 days with cyclophosphamide alone. A 1-h period of breathing air between exposure to halothane and administration of cyclophosphamide abolished the increased lethality of cyclophosphamide caused by halothane preexposure. Later exposure to 0.5% halothane for 2 h or for 20 h after giving cyclophosphamide, in addition to a prior 2-h exposure, further decreased the number of surviving animals.

Antitumor activity

Exposure to 0.5% halothane for 2 h and for 18 h after administration of cyclophosphamide had no effect on antitumor activity of cyclophosphamide against murine leukemia P388, as is shown in Table 3.

Organ toxicities

Studies were conducted to determine the cause of death of animals that received cyclophosphamide and halothane. The decrease in total white blood cell counts produced by cyclophosphamide was not significantly altered by postexposure of mice to 0.5% halothane for 2 h (Fig. 2). Other organ toxicities were studied on the 2nd or 3rd day after a combination of cyclophosphamide, 100 and 350 mg/kg, and subsequent 2-h halothane exposure at doses which would have resulted in death of the animals on day 5 or shortly thereafter. There was no sign of hepatic toxicity produced by cyclophosphamide or halothane, either alone or together, as indicated by the lack of any increase in serum total bilirubin and serum glutamate pyruvate transaminase (Table 4). There was a two-fold increase in serum BUN produced by halothane alone, but no further increase in animals receiving cyclophosphamide. This suggests that the mild renal toxicity apparently caused by halothane did not contribute to the acute death of the animals. The increase in bladder weight produced by cyclophosphamide was not significantly enhanced, and may in fact have been decreased by exposure to halothane, although the decrease was not statistically significant (Table 5).

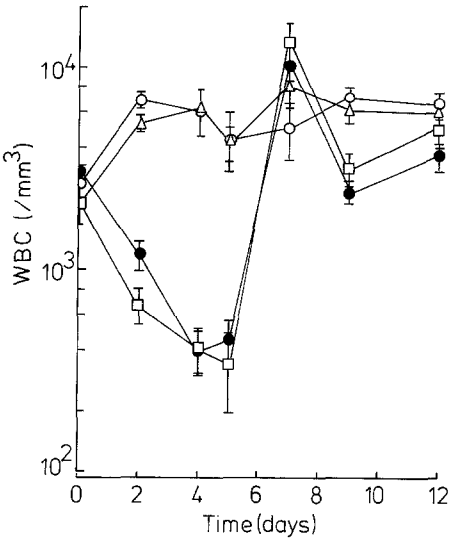

Fig. 2. Effect of halothane exposure on total WBC following administration of cyclophosphamide. Groups of four mice received cyclophosphamide IP at 100 mg/kg and were then exposed to 0.5% halothane in air for 2 h. Sequential total WBC counts were made on each animal. Values are means and *bars*, SEM. ○, no treatment; △, 0.5% halothane; ■, cyclophosphamide 100 mg/kg; ●, cyclophosphamide 100 mg/kg and 0.5% halothane

Table 4. Hepatic and renal toxicity of cyclophosphamide and halothane

	Bilirubin mg/100 ml	SGPT IU/l	BUN mg/100 ml
Control	0.37 ± 0.12	25.0 ± 7.2	9.6 ± 1.5
Cyclophosphamide	0.53 ± 0.13	23.5 ± 6.1	8.8 ± 1.8
Halothane	0.54 ± 0.11	25.3 ± 7.6	18.9 ± 3.4[a]
Cyclophosphamide + halothane	0.59 ± 0.16	28.4 ± 10.2	19.4 ± 8.6[a]

Groups of mice received cyclophosphamide, 350 mg/kg, IP and/or were exposed to 0.5% halothane for 2 h. Serum total bilirubin, serum glutamate-pyruvate transaminase (SGPT), and blood urea nitrogen (BUN) were measured 72 h later. Values are means ± SEM

[a] $P < 0.05$ against control

Table 5. Effect of halothane exposure on bladder toxicity of cyclophosphamide

	Bladder weight without halothane (mg/100 g body weight)	Bladder weight with halothane (mg/100 g body weight)
Control	102 ± 6	102 ± 5
Cyclophosphamide		
100 mg/kg	167 ± 21[a]	151 ± 11[a]
350 mg/kg	329 ± 47[a]	263 ± 9[a]

Values are means ± SEM for five mice

[a] $P < 0.05$ against appropriate non-cyclophosphamide-treated control

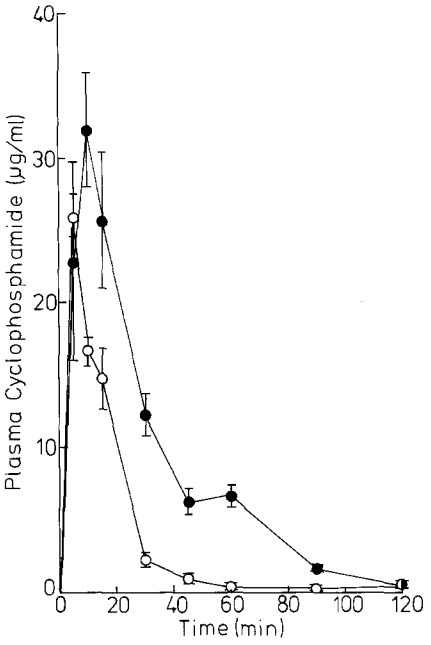

Fig. 3. Effect of halothane on plasma cyclophosphamide. Groups of four mice received cyclophosphamide, 65 mg/kg IP O, control mice; ●, mice preexposed to halothane for 5 min after cyclophosphamide; *bars*, SEM

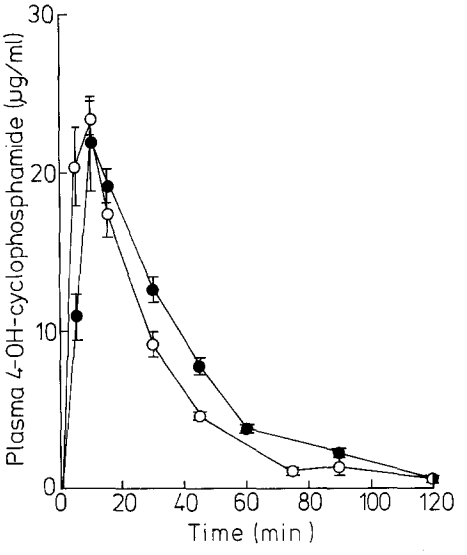

Fig. 4. Effect of halothane exposure on plasma 4-hydroxycyclophosphamide. Groups of four mice cyclophosphamide received 65 mg/kg IP. O control mice; ● mice preexposed to halothane for 5 min after cyclophosphamide; *bars*, SEM

Pharmacokinetics

Plasma concentrations of cyclophosphamide following a nonlethal dose of cyclophosphamide in control mice and in mice exposed to 0.5% halothane are shown in Fig. 3. Plasma concentrations of cyclophosphamide were consistently higher in halothane-exposed than in control mice. Elimination of plasma cyclophosphamide in both groups of mice was biphasic, with a $t_{1/2}\alpha$ of 5.6 min and a $t_{1/2}\beta$ of 20.4 min in control mice and a $t_{1/2}\alpha$ of 5.2 min and a $t_{1/2}\beta$ of 21.7 min in halothane-exposed mice. Total-body plasma clearance of cyclophosphamide was reduced in halothane-exposed mice to 60.1 ml/min/kg, as against 188.5 ml/min/kg in control mice. The results suggest that halothane exposure caused a decrease in the apparent volume of distribution for cyclophosphamide. Plasma concentrations of 4-hydroxycyclophosphamide rose more slowly but achieved a similar peak concentration in halothane-exposed and control mice (Fig. 4). The area under the plasma concentration-time curve (AUC) for 4-hydroxycyclophosphamide over 2 h in halothane-exposed mice was 114% that in non-halothane-exposed mice.

Penetration of cyclophosphamide into brain

It was observed that mice treated with cyclophosphamide and exposed to halothane, but not mice treated with cyclophosphamide alone, exhibited a defective righting reflex, which resulted in the animal spinning around the long axis of the body when placed on its back. This suggested a possible central nervous system toxicity of the combination of halothane and cyclophosphamide. Halothane has been reported to affect the integrity of the blood-brain barrier [23]. We therefore studied the ability of cyclophosphamide and its metabolites to penetrate the brain of halothane-exposed mice. The effect of halothane exposure on levels of cyclophosphamide and polar metabolite in brain following IP administration of [ring-4-^{14}C]-cyclophosphamide is shown in Table 6. Plasma cyclophosphamide determined

Table 6. Effect of halothane exposure on brain levels of cyclophosphamide and metabolites

	Brain weight (% body weight)	Cyclophosphamide		Polar-metabolites	
		Plasma µg/ml	Brain µg/g	Plasma µg/ml	Brain µg/g
Control	1.97 ± 0.12	22.3 ± 1.0 (8.5 ± 2.9)	9.9 ± 0.8 (7.0 ± 2.9)	32.9 ± 1.3	1.8 ± 0.4
Halothane	2.02 ± 0.04	30.3 ± 0.8[a] (13.7 ± 1.5)[a]	14.2 ± 0.7[a] (14.3 ± 6.0)[a]	32.2 ± 1.4	2.9 ± 0.6

Groups of six mice received IP injections of [ring-4-^{14}C]-cyclophosphamide 65 mg/kg, 4 µCi/mouse. One group was exposed to 0.5% halothane in air. Mice were killed 20 min later and plasma and brain radioactive cyclophosphamide and nonpolar metabolites determined as described in the text. Values are means ± SEM. Values in parenthesis are parent cyclophosphamide determined by a specific gas chromatographic assay. Polar metabolites are expressed as cyclophosphamide equivalents

[a] $P < 0.01$

Table 7. Activity of cyclophosphamide and halothane against intracerebral leukemia P388

	Survival (days)		
	Without halothane	With halothane exposure for	
		2 h	20 h
Control	8.5	8.5	7.5
Cyclophosphamide			
30 mg/kg	8.5	12	–
90 mg/kg	12	13	12

Groups of six mice were inoculated intracerebrally with 10^6 leukemia P388 cells. At 24 h later cyclophosphamide was administered at 30 or 90 mg/kg and mice exposed to 0.5% halothane in air for 2 or 20 h. Survival times are expressed as median values.

[a] $P < 0.05$ against control in the absence of cyclophosphamide

[b] $P < 0.05$ against cyclophosphamide alone in treated animals (Mann-Whitney)

as solvent-extractable radioactivity gave higher values than cyclophosphamide determined by a specific gas chromatographic assay. This is probably because the organic solvent extraction procedure extracted some radioactive cyclophosphamide metabolites in addition to cyclophosphamide. There was no difference between cyclophosphamide in brain determined by the radioactive and gas chromatographic methods, probably because cyclophosphamide metabolites are relatively excluded from the brain [13]. In the present study, 20 min after IP administration of cyclophosphamide the ratio of brain to plasma cyclophosphamide was 0.44 but the ratio of brain to plasma polar cyclophosphamide metabolites, only 0.05. Halothane exposure increased plasma cyclophosphamide concentrations by 36% and brain cyclophosphamide by 43%, but had no significant effect on the level of cyclophosphamide metabolites in either plasma or brain. The effect of halothane exposure on the antitumor activity of IP cyclophosphamide against intracerebral leukemia P388 tumor is shown in Table 7. Halothane alone had no effect on the survival mice of intracerebral inoculated with P388 and did not significantly alter the increased survival produced by IP cyclophosphamide.

Discussion

Exposure of mice to 0.5% halothane in air, which is half the reported MAC for halothane [10] and close to the maintenance value for clinical anesthesia, for times as short as 2 h produced an increase in the lethality of cyclophosphamide. The 30-day LD_{50} of cyclophosphamide in control mice was 251 mg/kg and that in mice exposed to halothane for 2 h, 152 mg/kg. Exposure of mice to halothane for 20 h did not produce a further increase in the lethality of cyclophosphamide, and the LD_{50} was 158 mg/kg. The median survival time of mice treated with cyclophosphamide at doses between 137 and 240 mg/kg was more than 30 days in the absence of halothane, 12 days with 2 h halothane exposure, and10.5 days with 20 h halothane exposure. Survival of mice was decreased irrespective of whether 2-h halothane exposure preceded or followed cyclophosphamide administration, but was abolished by a 1-h interval of breathing air between earlier halothane exposure and cyclophosphamide administration. These results suggest that lethality is only increased when both agents are present together.

The reason for the increased lethality of cyclophosphamide and halothane in mice is not known. The increase in the slope of the cyclophosphamide lethality curve in mice exposed to halothane suggests a different mechanism of lethality in halothane-exposed mice than in mice receiving cyclophosphamide alone. There does not appear to be a pharmacokinetic interaction between the agents, at least at sublethal doses of cyclophosphamide, that could account for the increased lethality. Halothane [13] and cyclophosphamide [28] are both substrates for hepatic microsomal cytochrome P-450, and exposure of mice to 0.5% halothane can decrease in vivo cytochrome P-450-dependent metabolism [19]. Although plasma clearance of cyclophosphamide at a nonlethal dose of cyclophosphamide was decreased by 68% in mice exposed to halothane, plasma levels of 4-hydroxycyclophosphamide, the first product of cyclophosphamide metabolism [13] were not changed. 4-Hydroxycyclophosphamide is the plasma transport form of activated cyclophosphamide, which releases phosphoramide mustard, the ultimate alkylating species [7]. The duration of exposure to a threshold concentration of 4-hydroxycyclophosphamide is thought to be critical for the cytotoxic activity of cyclophosphamide [15]. Aldehyde dehydrogenase, the enzyme responsible for elimination of 4-hydroxycyclophosphamide [8], is inhibited by other halogenated hydrocarbons [17], and may be inhibited by halothane, which could account for the unchanged peak concentration and AUC for plasma 4-hydroxycyclophosphamide despite a decreased rate of formation from cyclophosphamide.

The white blood cell nadir and antitumor activity of cyclophosphamide against P388 leukemia, both measures of cyclophosphamide cytotoxicity, were not affected by halothane exposure. This agrees with the lack of change in 4-hydroxycyclophosphamide plasma concentrations. It should be noted that both white blood cell nadir and antitumor activity were measured at sublethal doses of cyclophosphamide. Other specific toxicities of cyclophosphamide were measured after lethal doses of cyclophosphamide. Hemorrhagic cystitis is a potentially lethal effect of cyclophosphamide, and is caused by acrolein released during hepatic microsomal metabolism of cyclophosphamide [1]. Bladder toxicity was not increased by halothane exposure, and mice even appeared to be protected to a small degree against cyclophosphamide bladder toxicity by halothane exposure. In some conditions, halothane can cause

acute hepatic toxicity in experimental animals [28]. However, no evidence could be found for hepatotoxicity caused by halothane and cyclophosphamide, either alone or in combination. There was also no evidence for increased renal toxicity of halothane with cyclophosphamide. Because we observed that mice exposed to halothane receiving cyclophosphamide appeared to exhibit central nervous system toxicity, studies were conducted on the penetration of cyclophosphamide and its polar metabolite into the brain. Volatile anesthetics such as halothane have been reported to enhance the uptake of compounds across the blood-brain barrier [23]. Halothane exposure had no effect on the penetration of cyclophosphamide or its polar metabolites into brain, whether measured directly or as the activity of IP cyclophosphamide against intracerebral P388 tumor. Reported toxicities of cyclophosphamide that were not studied but might have contributed to the death of the animals were cardiotoxicity [16, 25] and lung toxicity [6]. If cyclophosphamide lung injury is caused by acrolein, as has been suggested [24], it might be expected to be unaffected by halothane exposure, like bladder toxicity.

The significance of the increased lethality of cyclophosphamide observed in the presence of halothane in mice for patients receiving the agents is not known. Halothane is still used as an anesthetic agent although it produces hepatic necrosis. However, the incidence of this toxicity is low in adults and essentially nonexistent in children [3]. Therefore, the report of fatal hepatic necrosis in a child with neuroblastoma who received halothane anesthesia followed by intensive chemotherapy with cyclophosphamide, vincristine, and dacarbazine [27] suggests that the combination of anticancer drugs with halothane is responsible for this toxicity. In the same study fatal hepatic necrosis was observed in a young adult receiving enflurane anesthesia and high-dose methotrexate chemotherapy, together with other cases of nonfatal hepatic injury in children and young adults receiving enflurane or halothane anesthesia and intensive chemotherapy. Halothane anesthesia has recently been implicated at our institution as a cause of hepatic encephalopathy in a child with acute lymphoblastic leukemia receiving vincristine and daunomycin (Dr. L. A. Christianson, Department of Anesthesiology, personal communication). In the present study we found no indication of liver toxicity with the combination of halothane and cyclophosphamide. The LD_{50}, although not the LD_{10}, for cyclophosphamide was decreased by exposure of mice to halothane. If the same dose-response relationship holds in human as in mice, patients receiving cyclophosphamide at the maximum tolerated dose, which is often equated to the LD_{10} in mice [11], would not be expected to be subject to a lethal interaction when exposed to halothane. This does not mean, however, that there might not be an increase in a sublethal organ toxicity in patients exposed to a combination of cyclophosphamide and halothane; since we have failed to identify the target organ for the increased lethality in mice, however, this is a difficult question to answer.

In summary, we have shown that mice exposed to the volatile anesthetic agent halothane at concentrations that might be encountered in humans during surgical anesthesia show an increased sensitivity to the lethal effects of the anticancer and immunosuppressive drug cyclophosphamide. We have been unable to identify a particular organ as the site of increased toxicity leading to death of the animals. These observations, combined with reports of an apparently increased incidence of toxic effects in patients given cancer chemotherapeutic agents in close proximity to general anesthesia, should alert physicians and others to the possibility of an interactions between volatile anesthetic agents and chemotherapeutic drugs.

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References

1. Brock N, Stekar J, Pohl J, Niemeyer U, Scheffler G (1979) Acrolein, the causative factor of uriotoxic side-effects of cyclophosphamide, ifosfamide, trofosfamide and sulfosfamide. Arzneimittelforsch 29: 659
2. Bruce D (1973) Anesthetic-induced increase in murine mortality from cyclophosphamide. Cancer 31: 361
3. Carney FM, Van Dyke RA (1972) Halothane hepatitis: A critical review. Anesth Analg 51: 135
4. Chabner BA (1982) The role of drugs in cancer treatment. In: Chabner BA (ed) Pharmacologic principles of cancer treatment. Saunders, Philadelphia, pp 3–14
5. Chung F (1982) Cancer, chemotherapy and anesthesia. Can Anaesth Soc J 29: 364
6. Collins CH, Wilson CM, Jones JM (1980) Cyclophosphamide-induced lung damage in mice: protection by a small preliminary dose. Br J Cancer 41: 901
7. Domeyer BE, Sladek NE (1980a) Kinetics of cyclophosphamide transformation in vivo. Cancer Res 40: 174
8. Domeyer BE, Sladek NE (1980b) Metabolism of 4-hydroxycyclophosphamide/aldophosphamide in vitro. Biochem Pharmacol 29: 2903
9. Dorr RT, Fritz WL (1980) Drug interactions. In: Cancer chemotherapy handbook. Elsevier, New York, pp 75–97
10. Eger EI, Saidman CJ, Brandstater B (1965) Minimum alveolar anesthetic concentration: A standard of anesthetic potency. Anesthesiology 26: 756
11. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966) Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50: 219
12. Geran RI, Greenberg NH, MacDonald MM, Schumacher AM, Abbott BJ (1972) Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother Rep [3] 3: 1
13. Grochow LB, Colvin M (1979) Clinical pharmacokinetics of cyclophosphamide. Clin Pharmacol 4: 380
14. Hacker MP, Newman RA, Ershler WB (1982) The prevention of cyclophosphamide-induced cystitis in mice by disulfiram. Res Commun Chem Pathol Pharmacol 35: 145
15. Harris RN, Basseches PJ, Appel PL, Durski AM, Powis G (1984) Carbon tetrachloride-induced increase in the antitumor activity of cyclophosphamide in mice: A pharmacokinetic study. Cancer Chemother Pharmacol 12: 167
16. Hermann EH, Mhatre RM, Waravdekar VS, Lee IP (1972) Comparison of the cardiovascular actions of NSC-109,724 (Isosfamide) and cyclophosphamide. Toxical Appl Pharmacol 23: 178
17. Hjelle JJ, Peterson DR (1981) Decreased in vivo acetaldehyde oxidation and hepatic aldehyde dehydrogenase inhibition in C57BL and DBA mice treated with carbon tetrachloride. Toxicol Appl Pharmacol 59: 15
18. Juma FP, Rogers HJ, Trounce JR (1980) The pharmacokinetics of cyclophosphamide, phosphoramide mustard and Nor-nitrogen mustard studied by gas chromatograph in patients receiving cyclophosphamide therapy. Br J Clin Pharmacol 10: 327

19. Kooistra KL, Moses CJ, Powis G, Van Dyke RA (1984) The influence of halothane, enflurane and isoflurane on antipyrine pharmacokinetics in mice. Fed Proc 43: 338
20. Mellet LB, El Dareer SM, Rall DP, Adamson RH (1969) Metabolism of cyclophosphamide-C^{14} by various marine species. Arch Int Pharmacodyn 177: 60
21. Metzler CM, Elfring G, McEwen EJ (1974) A package of computer programs for pharmacokinetic modeling. Biometrics 30: 562
22. Migdalof BH (1976) Methods for obtaining drug time course data from individual small laboratory animals: serial microblood sampling and assay. Drug Metab Rev 5: 295
23. Nemoto EM (1979) Blood-brain barrier transport during anesthesia. Adv Exp Med Biol 131: 167
24. Patel JM, Block ER, Hood CI (1984) Biochemical indices of cyclophosphamide-induced lung toxicity. Toxicol Appl Pharmacol 76: 128
25. Slavin RE, Millan JC, Mullins GM (1975) Pathology of high-dose intermittent cyclophosphamide therapy. Hum Pathol 6: 693
26. Snedecor GW, Cochran WG (1967) Statistical methods, 6th edn. Iowa State University, Ames
27. Spiegel RJ, Pizzo PH, Fantone JC, Zimmerman HJ (1980) Fatal hepatic necrosis after high-dose chemotherapy following haloalkane anesthesia. Cancer Treat Rep 64: 1023
28. Van Dyke RA (1982) Hepatic centrilobular necrosis in rats after exposure to halothane, enflurane, and isoflurane. Anesth Analg 61: 812
29. Van Dyke RA, Powis G (1983) Potentiation of the toxicity of cyclophosphamide and BCNU in mice by the general anesthetic halothane. Proc Am Assoc Cancer Res 24: 319
30. Voelcker R, Hohorst HJ (1979) Fluorometrische Bestimmung von "aktiviertem" Cyclophosphamid und Ifosfamid im Blut. J Cancer Res Clin Oncol 93: 233

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