INHIBITORY EFFECTS OF CORTICOIDS ON REDUCTIVE HALOTHANE DEHALOGENATION

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SUMMARY

The effects of corticoids, hydrocortisone, methylprednisolone, betamethasone and dexamethasone, on the reductive metabolism of halothane to produce chloro-difluoroethylene (CDE) and chlorotrifluoroethane (CTE) in the liver microsomes of guinea-pig were examined. The substrate differential spectrum for methylprednisolone showed a peak at 412 nm and trough at 395 nm, typical modified type II. The other corticoids showed a similar spectral change. The corticoids had no effect on NADPH-cytochrome P450 reductase. All of these corticoids inhibited the reductive dehalogenation of halothane. The concentrations of hydrocortisone, methylprednisolone, betamethasone and dexamethasone causing 50% inhibition of CDE formation from halothane were 5.1± 0.7 mg/ml, 3.1±1.2 mg/ml, 2.3±0.4 mg/ml and 2.4 ±0.6 mg/ml, respectively, with no significant differences except for hydrocortisone. The concentrations of hydrocortisone, methylprednisolone, betamethasone and dexamethasone causing 50% inhibition of CTE formation from halothane were 4.9±0.5 mg/ml, 3.1±1.0 mg/ml, 2.4±0.3 mg/ml and 28.0±9.1 mg/ml, respectively, with no significant differences except for dexamethasone. Our results showed that corticoids inhibit halothane metabolism.

KEY WORDS

corticoids, halothane, inhibition, interaction, metabolism

INTRODUCTION

Halothane, 2-bromo-2-chloro-1,1,1-trifluoroethane, is used as a clinical anesthetic in many countries. Halothane is metabolized by the liver microsomal mixed function oxidase system through two pathways. One is the reductive pathway in which two reductive metabolites, chloro-difluoroethylene (CDE) and chloro-trifluoroethane (CTE), are produced and eliminated in the expiratory gas. The other is an oxidative pathway in which trifluoroacetic acid is produced and eliminated in the urine. Both reactions require NADPH as the electron donor. Under the reductive pathway, a radical intermediate is produced, and may lead to lipid peroxidation and liver necrosis. Some studies showed that reductive intermediates of halothane can directly and indirectly damage the liver by peroxidative decomposition of fatty acids in the phospholipid portion of cell membranes, with subsequent damage to vital intracellular structures /1-3/. The radical metabolites, such as CF_3CHC1, produced by reductive metabolism of halothane may cause lipid peroxidation /4-6/.

Hepatic microsomal drug metabolism has been reported to be inhibited by diltiazem and verapamil /7/. Corticoids are also degraded by the liver microsomal mixed function oxidase system containing cytochrome P450. It would be expected that corticoids might interfere with halothane metabolism. If corticoids affect the cytochrome P450 system and thus inhibit halothane metabolism, corticoids may prevent halothane induced liver injury caused by reductive metabolism. In this study we examined whether corticoids affect reductive halothane dehalogenation by the liver microsomes of guinea-pigs.

MATERIALS AND METHODS

This study was carried out according to the Guide on Animal Experimentation in Research Facilities for Laboratory Animal Science, School of Medicine, Hiroshima University.

Reagents

Analytical grade potassium hydroxide, sodium hydroxide, calcium hydroxide and other reagents which are commercially available were used. Halothane was obtained from Dynabot Pharmaceutical Co. (Tokyo, Japan). Dexamethasone sodium phosphate and hydrocorti-

sone sodium phosphate were generous gifts from Banyuu Pharmaceutical Co. Ltd. (Tokyo, Japan). Hydrocortisone hemisuccinate and methyl prednisolone sodium succinate were generous gifts from Japan Upjohn Ltd. (Tokyo, Japan). Betamethasone sodium phosphate was a generous gift from Shionogi Pharmaceutical Co. (Tokyo, Japan).

Animals

Male Hartley guinea-pigs with body weights of 225-275 g were used. The animals were killed after being starved for 24 hours followed by excision of the liver. After perfusing the livers through the portal vein with ice-cold physiological saline, the livers were homogenized in 0.05 M potassium phosphate buffer. Following centrifugation of the liver homogenate at 9,000 *g*, the supernatant was centrifuged at 105,000 *g*. The resulting sediment was suspended in 0.1 M potassium phosphate buffer and used as the liver microsome suspension.

Reaction system and measurements

The microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.4). A sample (0.9 ml) of the microsomal suspension from 0.1 g wet liver was sealed in a 12.5 ml test tube. The test tube was then perfused with deoxygenized nitrogen for 10 min. A 0.1 ml sample of potassium phosphate buffer (control) or 0.1 ml of corticoid solutions (final concentration: 0.08-10 mg/ml) was added. These suspensions were incubated for 5 min at 37°C while being shaken 100 times per minute. Another incubation was started and kept for 10 min under the same conditions after the addition of 0.1 ml of 30 mM NADPH and halothane (final concentration: 1 mM) was added. After 10 min, 0.5 ml gas phase was analyzed on a HP-5890 Series II (Hewlett Packard, USA) gas chromatograph equipped with a flame ionization detector. The reductive metabolites of halothane, chloro-trifluoroethane (CTE) and chloro-difluoroethylene (CDE), were measured following Fujii's method /8/. Reciprocals of each reaction rate were plotted against the concentration of steroids. Concentration of corticoids causing 50% inhibition of CDE and CTE formation were determined from the intersection of the regression line with the x-axis. Cytochrome P450 was measured by Omura's and Sato's method /9/. NADPH-cytochrome P450 reductase was assayed by the methods of Omura /10/. The amount of protein was measured using the method of Lowry *et al.* /11/.

Statistical verification

ANOVA and Student's t-test were used for statistical analysis of the results. Differences with a p-value of less than 0.05 were regarded as significant.

RESULTS

For a corticosteroid to affect reductive dehalogenation of halothane, it must interfere with cytochrome P450. A substrate differential spectrum for methylprednisolone showed a peak at 412 nm and trough at 395 nm, typical modified type II. The other corticoids also showed a similar spectral change. These spectral changes reached a plateau after 10 min. These findings indicate that the corticoids combine with the heme part of cytochrome P450.

Effect of corticoids on NADPH-cytochrome P450 reductase

The electron transport system consisted of NADPH-cytochrome P450 reductase and cytochrome P450. We examined the effect of the corticoids on NADPH-cytochrome P450 reductase. The corticoids used in this experiment, hydrocortisone, methylprednisolone, betamethasone and dexamethasone, did not affect NADPH-cytochrome P450 reductase (data not shown).

Effects of corticoids on CDE and CTE formation (Fig. 1)

To determine the effect of the corticoids on reductive dehalogenation of halothane, the concentrations inhibiting the production of CDE and CTE by 50% were calculated for 1 mM halothane, which is near the anesthetic concentration. All the corticoids in this study inhibited reductive dehalogenation of halothane. The concentrations of hydrocortisone, methylprednisolone, betamethasone and dexamethasone causing 50% inhibition of CDE formation from halothane were 5.1±0.7 mg/ml, 3.1±1.2 mg/ml, 2.3±0.4 mg/ml and 2.4±0.6 mg/ml, respectively. There were no significant differences among the corticoids except for hydrocortisone. The concentrations of hydrocortisone, methylprednisolone, betamethasone and dexamethasone causing 50% inhibition of CTE formation from halothane were 4.9±0.5 mg/ml, 3.1±1.0 mg/ml, 2.4±0.3 mg/ml and 28.0±9.1 mg/ml, respectively.

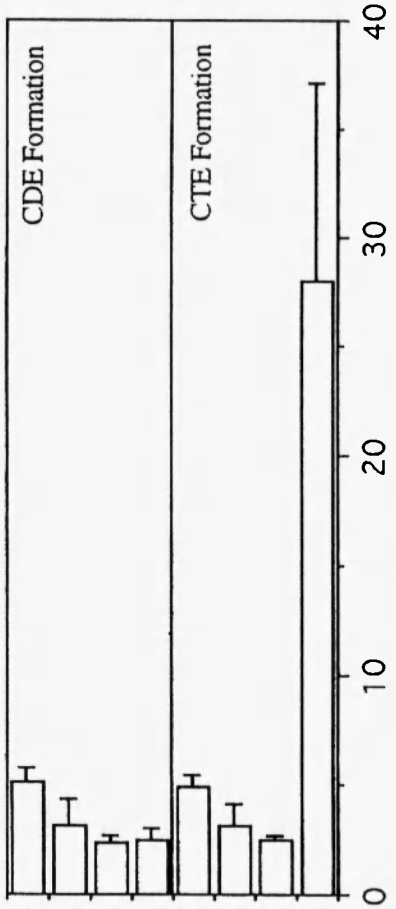

Fig. 1: Effect of corticoids on CDE and CTE formation. Concentrations of 50% inhibition for production of CDE and CTE were calculated using 1 mM halothane which is near the anesthetic concentration. The concentrations of hydrocortisone, methylprednisolone, betamethasone and dexamethasone causing 50% inhibition of CDE formation from halothane were 5.1±0.7 mg/ml, 3.1±1.2 mg/ml, 2.3±0.4 mg/ml and 2.4±0.6 mg/ml, respectively. There were no significant differences among the corticoids except for hydrocortisone. The concentrations of hydrocortisone, methylprednisolone, betamethasone and dexamethasone causing 50% inhibition of CTE formation from halothane were 4.9±0.5 mg/ml, 3.1±1.0 mg/ml, 2.4±0.3 mg/ml and 28.0±2.1 mg/ml, respectively. There were no significant differences among the corticoids used in this experiment except for dexamethasone.

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DISCUSSION

The substrates of cytochrome P450 can be classified into Type I, Type II and modified Type II. A Type I substrate combines with the protein part of cytochrome P450, the Type II and modified Type II substrates combine with the heme part of cytochrome P450. The substrate differential spectrum for the corticoids used in this study was typical of that belonging to a modified Type II substrate. The substrate differential spectrum for halothane was that of a Type I substrate, but changed to that of a modified Type II substrate after reduction by NADPH under anaerobic conditions. Halothane undergoes dehalogenation to produce CDE and CTE by liver microsomal P450 under anaerobic conditions /8/. Corticoids are also degraded by the liver microsomal electron transport system including cytochrome P450. In this study, we clearly showed that the corticoids inhibited anaerobic dehalogenation of halothane. Corticoids may compete with halothane on the heme part of cytochrome P450.

Under the reductive pathway, a radical intermediate is produced, and may lead to lipid peroxidation and liver necrosis. The radical metabolites, such as CF_3CHCl, produced by reductive metabolism of halothane may cause lipid peroxidation /4-6/. Our findings indicate that corticoids may prevent halothane-induced liver injury caused by reductive metabolism. However, because the concentrations of steroids causing 50% inhibition were higher than normal plasma concentrations, clinical doses of steroids would not prevent halothane-induced liver injury.

CONCLUSION

Corticoids interfere with the binding of halothane to the heme part of cytochrome P450, and inhibit reductive halothane dehalogenation.

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