EFFECT OF DOXAPRAM ON THE ACTION OF OTHER DRUGS AND THE HEPATIC DRUG-METABOLIZING SYSTEM IN MICE

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Abstract—Effects of doxapram, a respiratory stimulant, on the action of other drugs and the activity of the hepatic drug-metabolizing enzyme were studied in mice. The hypothermic effect induced by aminopyrine and the muscle relaxative effect induced by meprobamate were potentiated by the pretreatment with doxapram 60 min before. Furthermore, doxapram significantly enhanced the lethalities of picrotoxin and strychnine and the analgesic actions of aminopyrine and morphine. The plasma concentration of aminopyrine or pentobarbital in doxapram-treated mice was higher than those in untreated mice, and the plasma concentration of normustard related to an active metabolite of cyclophosphamide after the administration of cyclophosphamide was lower in doxapram-treated mice. On the other hand, doxapram (50 mg/kg, i.p.) reduced remarkably the activities of aminopyrine N-demethylase and aniline hydroxylase in the hepatic 9,000×g supernatant fraction, and also reduced the cytochrome P-450 contents in hepatic microsomes. However, no significant alteration by doxapram was observed on the activities of NADH-ferricyanide reductase and NADPH-cytochrome c reductase and cytochrome b5 contents. It seems likely that the mechanisms of the interaction between doxapram and combined drugs involved the depression of the hepatic drug-metabolizing system in microsomes and a subsequent variation of drug level in the plasma.

Doxapram has been reported to be a potent respiratory stimulant (1, 2) and to produce marked arousal effects in barbiturate-induced sleep (3–6). However, it was shown earlier that doxapram potentiated the hypnotic action induced by amobarbital and pentobarbital in rats (7) and mice (8).

Previously, we demonstrated that pentobarbital sleeping time was significantly prolonged 60 min after the intraperitoneal administration of doxapram in mice and that the level of pentobarbital in plasma was higher than that after the administration of pentobarbital alone (9). We found also that doxapram had little influence on the sensitivity of the central nervous system to pentobarbital (9).

The drug interaction is related to changes of drug metabolism in the majority of cases. It was known that the action and toxicity of some combined drugs were changed by SKF-525A, a representative drug which is a hepatic enzyme inhibitor (10). Although doxapram is a respiratory stimulator, it has an inhibitory action on the hepatic enzyme that is similar to SKF-525A. Therefore, the effect of doxapram on the actions of combined drugs is of interested in connection with the
drug metabolism.

The present investigation was designed to determine whether there were some interactions between doxapram and the other drugs in mice and to clarify possible mechanisms for this interaction. The drugs which had activities that were easily changed by variations of their metabolisms were selected for combination with doxapram.

MATERIALS AND METHODS

Drugs: The drugs used were obtained from the following commercial sources: Doxapram [1-ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidinone hydrochloride hydrate] from Kissei Yakuhin Co., pentobarbital sodium and hexobarbital sodium from Tokyo Kasei Co., thiopental sodium from Tanabe Seiyaku Co., SKF-525A [2’-(diethylamino) ethyl-2,2-diphenylvalerate hydrochloride] from Smith Kline and French Laboratories, meprobamate from Daiichi Seiyaku Co., aminopyrine from Yamada Seiyaku Co., morphine hydrochloride from Sankyo Co., cyclophosphamide from Shionogi Seiyaku Co., strychnine nitrate and picrotoxin from Wako Junyaku Kogyo Co. All drugs for injection were dissolved in physiological saline and intraperitoneally administered.

Animals: Male ddY mice (20-22 g) were purchased from the Animal Center of Shizuoka Experimental Laboratories. Food (Clea CE-2, Clea Japan Co.) and water were provided ad libitum. The breeding were carried out at 22-24°C.

Sleeping time: Sleeping time was defined as the time between the loss and the recovery of the righting reflex.

Body temperature: Rectal temperatures of mice were measured by a thermometer with a thermister element (Shibaura Electronics, MGA-3). Mice having body temperatures of 37-38°C were employed in this experiment after selection by measuring twice at 15 min intervals.

Muscle relaxative action: Muscle relaxative actions in mice were examined by the rotarod test (11) and the traction test (12).

Convulsive action: The time from appearance of convulsion to death induced by strychnine (0.9-2 mg/kg) or picrotoxin (7.5-30 mg/kg) was measured.

Analgesic action: Analgesic action of aminopyrine or morphine was examined by the pressure method (13).

Tumor cells: Ehrlich ascites carcinoma cells maintained in the peritoneum of ddY mice were used. Seven days after the cell inoculation (1 x 10⁶/mouse), the cells were collected from the mice.

Antitumor activity: Cyclophosphamide was injected once intraperitoneally at 24 hr after inoculation of tumor cells (1 x 10⁶ cells) into the right thigh. The antitumor activity of cyclophosphamide was estimated by comparison with the control by weighing the solid tumor on the 15th day after cell inoculation.

Determination of the level of hexobarbital, pentobarbital, cyclophosphamide, and aminopyrine in plasma: Hexobarbital (80 mg/kg) or pentobarbital (50 mg/kg) was intraperitoneally administered, and the plasma was collected 30 min after the injection of the barbiturate. Cyclophosphamide or aminopyrine was intraperitoneally administered at 100 mg/kg, and the plasma was collected at the indicated time. Doxapram was administered 60 min before these four drugs. The observations on the doxapram-pretreated group and the corresponding control were carried out at the same time under the same conditions. The level of hexobarbital, pentobarbital, and aminopyrine in the plasma were assayed by the methods of Cooper and Brodie (14), Brodie et al. (15), and Brodie and Axelrod (16), respectively. The metabolite of cyclophosphamide was estimated according to the method of Sladek (17).
Enzyme assays: The excised livers were placed in ice-cold 1.15% KCl solution. All further procedures for the preparation of the enzyme were carried out at 0–4°C. A 20% homogenate fraction was prepared in 1.15% KCl solution using a Potter-Elvehjem type homogenizer. The homogenate was transferred to polypropylene centrifuge tubes and centrifuged at 9,000×g for 20 min to obtain the 9,000×g supernatant fraction. Microsomal pellets were prepared by centrifuging the 9,000×g supernatant fraction in an ultracentrifuge at 105,000×g for 60 min at 4°C and suspending the resulting pellet in 0.05M phosphate buffer (pH 7.4) so that 1 ml of the suspension contained microsomes obtained from 200 mg of liver. The activities of aminopyrine N-demethylase and aniline hydroxylase in the 9,000×g liver supernatant fraction were assayed by a slight modification of the method of Kitagawa et al. (18). The activity of pentobarbital oxidase in the 9,000×g liver supernatant fraction was assayed by the method of Brodie et al. (15). Microsomal cytochrome P-450 and cytochrome b5 contents of the liver were determined according to the method of Omura and Sato (19) in a spectrophotometer (Shimazu UV 300). The activity of UDP-glucuronyl transferase in the 9,000×g liver supernatant fraction was determined by the modified method of Hollman and Touster (20). The activities of NADPH-cytochrome c reductase and NADH-ferricyanide reductase were determined by the method of Williams and Kamin (21) and Mihara and Sato (22), respectively.

Protein was determined by the method of Lowry et al. (23) with bovine serum albumin as the standard.

The statistical significance of the results was determined by the Student’s t-test.

RESULTS

Effect of doxapram treatment on sleeping action of hexobarbital or thiopental and the hypothermic action of aminopyrine: The mean sleeping time±S.E. of the intraperitoneal administration of hexobarbital (80 mg/kg) or thiopental (60 mg/kg) was 31.9±2.5 and 24.7±4.1 min, respectively. Hexobarbital and thiopental sleeping times of the groups with 25 mg/kg, i.p., of doxapram 60 min before were 105.4±10.1 and 61.6±10.2 min, respectively. Furthermore, the hypothermic action of aminopyrine (50 or 100 mg/kg, i.p.) in the group with 50 mg/kg, i.p., of doxapram 60 min before was stronger than that of the control group, continuing for a period of 90 min following aminopyrine.

Effect of doxapram treatment on the muscle relaxative action of meprobamate, the analgesic actions of aminopyrine and morphine, and the convulsive actions of strychnine and picrotoxin: As shown in Fig. 1, the muscle relaxative action of meprobamate (100 mg/kg, i.p.) was strongly potentiated by the pretreatment with doxapram (50 mg/kg, i.p.) 60 min before the examinations by the rotarod test and the traction test.

Furthermore, pretreatment with doxapram (50 mg/kg, i.p.) 60 min before enhanced the analgesic activities of aminopyrine (200 mg/kg, i.p.) and morphine (5 mg/kg, i.p.) as observed by the pressure method.

Figure 2 shows the effect of doxapram administered 60 min before on the convulsive action of two convulsants. Regarding the convulsion induced by picrotoxin (7.5 mg/kg, i.p.), the pretreatment of doxapram 60 min before picrotoxin caused significant acceleration of the onset of convulsion and death. Similarly, a potentiating effect of doxapram was seen in combination with strychnine: by the pretreatment with doxapram (50 mg/kg, i.p.), the lethality of strychnine at a dose of 0.9 mg/kg, i.p., or 2 mg/kg, i.p., considerably increased and the death time induced by strychnine at the same dose was shortened.
Effect of doxapram treatment on the antitumor activity of cyclophosphamide: The antitumor activity of cyclophosphamide on Ehrlich solid tumors in mice was examined alone and in combination with doxapram (Table 1). The mean tumor weight±S.E. of the control group on the 15th day after the cell inoculation was 7.00±0.28 g. A single dose of cyclophosphamide at 100 mg/kg, i.p., showed an inhibitory effect on tumor growth.

Fig. 1. Effect of doxapram on muscle relaxation induced by meprobamate [A] and on analgesic actions of aminopyrine and morphine [B] in mice. Each group was comprised of 10 mice. Doxapram (50 mg/kg) was injected intraperitoneally 60 min before the intraperitoneal injection of meprobamate (100 mg/kg), aminopyrine (200 mg/kg), or morphine (5 mg/kg). N1 and N2 in [B] indicate the first and second response, respectively, before the administration of analgesics. Symbols in [A] represent: (○): 100 mg/kg meprobamate alone, (□): 50 mg/kg doxapram plus 100 mg/kg meprobamate, (———): the rotarod test, (—-—-—): the traction test. In [B], the pressure method, symbols are: (●—●): 200 mg/kg aminopyrine alone, (□—□): 50 mg/kg doxapram plus 200 mg/kg aminopyrine, (●—●): 5 mg/kg morphine alone, (□—□): 50 mg/kg doxapram plus 5 mg/kg morphine.

Fig. 2. Effect of doxapram on strychnine- and picrotoxin-induced convulsion in mice. Convulsants were injected intraperitoneally 60 min after the injection of doxapram. Each point represents the mean±S.E. of 10 mice. (A): Each column represents the time from administration of convulsant to death. Figures in parentheses show number of dead mice/number of mice used. (B): Each column represents the time from appearance of convulsion to death. Figures in parentheses show number of mice with convulsions/number of mice used (left) and number of dead mice/number of mice used (right). **P<0.01: significantly different from the corresponding control group.
Table 1. Effect of pretreatment with doxapram on the antitumor activity of cyclophosphamide

| Pretreatment | Treatment | Tumor weight mean±S.E. (g) | Antitumor activitya (Inhibitory %) |
|--------------|-----------|---------------------------|-----------------------------------|
| Drugs        | Dose (mg/kg) | Drugs | Dose (mg/kg) | | |
| Control (Saline) | 100 | Doxapram | 100 | 7.00±0.28 | 0 |
| Doxapram | 100 | Cyclophosphamide | 100 | 7.08±0.38 | 0 |
| Doxapramb | 50 | Cyclophosphamide | 100 | 1.95±0.43 | 72 |
| Doxapramb | 100 | Cyclophosphamide | 100 | 1.93±0.17 | 72 |
| SKF-525Ac | 10 | Cyclophosphamide | 100 | 1.78±0.28 | 75 |
| Doxapramb | 100 | Cyclophosphamide | 100 | 3.23±0.32* | 54 |
| SKF-525Ac | 10 | Cyclophosphamide | 100 | 3.41±0.41* | 51 |
| Doxapramb | 100 | Cyclophosphamide | 100 | 3.29±0.53* | 53 |

Groups of 12 mice were used. Drugs were injected intraperitoneally. a) The antitumor activity was evaluated by weighing the solid tumor on the 15th day after Ehrlich ascites cell inoculation (1×10⁶ cells). Cyclophosphamide was injected once, 24 hr after the cell inoculation. b) Dose: 50 min before the injection of cyclophosphamide. c) Drugs were injected 30 min before the injection of cyclophosphamide. *P<0.05: significantly different from the corresponding control group.

Fig. 3. Effect of doxapram on the levels of pentobarbital, hexobarbital, aminopyrine and a metabolite of cyclophosphamide in plasma: At various time intervals after administration of doxapram (50 mg/kg, i.p.), the pentobarbital and hexobarbital concentrations in plasma were determined 30 min after the administration of pentobarbital (50 mg/kg, i.p.) or hexobarbital (80 mg/kg, i.p.). As shown in Fig. 3, a biphasic change in the levels of both drugs was observed: 1–4 hr after the administration of doxapram, the concentrations of pentobarbital and hexobarbital in plasma increased as compared with those of the control group; and 12–24 hr after doxapram, the concentrations of both drugs decreased. Thus, the alterations of both barbiturate levels in plasma occurred with a difference in the pretreatment time by doxapram.

On the other hand, the effect of doxapram treatment 60 min before on the plasma concentration of normustard expressed as an active metabolite of cyclophosphamide and aminopyrine is shown in Fig. 4: the plasma concentration of aminopyrine in doxapram-treated mice was higher than that in untreated mice. However, the plasma concentration of
normustard was lower in doxapram-treated mice.

Effect of doxapram on the hepatic drug-metabolizing system: Effect of doxapram on the activity of the drug-metabolizing enzyme in the hepatic 9,000×g supernatant fraction in mice was investigated and the results are shown in Fig. 5. The activities of aminopyrine N-demethylase, pentobarbital oxidase, and aniline hydroxylase decreased 60 min after the administration of doxapram (25, 50 or 100 mg/kg, i.p.); and the activity of UDP-glucuronyl transferase was slightly inhibited by the injection of doxapram. As shown in Fig. 6, doxapram (50 mg/kg, i.p.) reduced remarkably the activities of aminopyrine N-demethylase and aniline hydroxylase in the hepatic 9,000×g supernatant fraction, 1–24 hr and 1–4 hr later, respectively. Doxapram (50 or 100 mg/kg, i.p.) also reduced cytochrome P-450 contents in hepatic microsomes 1–4 hr later, but doxapram only slightly decreased the activities of NADPH-cytochrome c reductase and NADH-ferricyanide reductase and cytochrome b5 contents.

In contrast, after the consecutive administration of doxapram (50 mg/kg/day for 3 days or for 7 days, i.p.), the activity of aminopyrine N-demethylase was significantly higher than that of the control group, and the activities of aniline hydroxylase and NADPH-cytochrome
Fig. 6. Time course of the drug-metabolizing activity and contents of cytochrome P-450 and cytochrome b5 in hepatic microsomes after the injection of doxapram. Mice were sacrificed at a different time after treatment with doxapram. The activities of aminopyrine N-demethylase and aniline hydroxylase were determined in the hepatic 9,000 x g supernatant fraction. In panel [A]: Doxapram (50 mg/kg, i.p.) was administered. Symbols and control values were as follows: NADPH-cytochrome c reductase (○): 54.6±2.64 (nmol reduced/min/mg of protein), NADH-ferricyanide reductase (●): 331.5±4.60 (nmol reduced/min/mg of protein), amino-
pyrine N-demethylase (△): 1.78±0.05 (nmol/30 min/mg of protein), aniline hydroxylase (▲): 2.26±0.11 (nmol/30 min/mg of protein). In panel [B]: Doxapram at a dose of 50 mg/kg (—) or 100 mg/kg (-----), i.p. was admin-
istered. Symbols and control values were as follows: cytochrome P-450 (○): 0.96±0.05 (nmol/mg of protein) and cytochrome b5 (●): 0.36±0.02 (nmol/mg of protein). *P<0.05, **P<0.01: significantly different from the corresponding control group.

Fig. 7. Effect of the consecutive administration of doxapram on the drug-metabolizing system in the livers of mice. Control group was given saline solution (10 ml/kg, i.p.) instead of doxapram. The measurement of aminopyrine N-demethylase and aniline hydroxylase activities in the hepatic 9,000 x g supernatant fraction and NADPH-cytochrome c reductase and NADH-ferricyanide reductase activities and cytochrome P-450 and cytochrome b5 contents in hepatic microsomes were carried out at 24 hr after the final injection of 50 mg/kg/day of doxapram for 3 days (●) or for 7 days (■). *P<0.05, **P<0.01: significantly different from the corresponding control group.

c reductase and cytochrome P-450 contents were slightly higher than that of the control group. However, no effects on the activity of NADH-ferricyanide reductase and cyto-

DISCUSSION

In the present experiment, it was examined how doxapram affects the action of combined drugs by changing the hepatic microsomal drug-metabolizing system in mice.

It has been known that the actions of many drugs were often affected by the combination of drugs. The previous work in our laboratory showed that doxapram has a biphasic effect, prolongation at first and shortening later, on the pentobarbital sleeping time in mice (9). The barbiturates are oxidized by a hepatic microsomal enzyme (24), and the duration of sleep induced by the barbiturate is affected by stimulation or depression of the enzyme activity (25).

Pretreatment with doxapram 60 min before produced a significant increment of the hypnotic action induced by hexobarbital and thiopental. This result was similar to that
obtained when pentobarbital was administered with doxapram (9). The concentrations of pentobarbital and hexobarbital in plasma were markedly elevated 1–4 hr after the administration of doxapram and lowered 12–24 hr after as compared with those of untreated mice. Thus, the biphasic effect of doxapram as mentioned previously was also observed on the plasma levels of both drugs. Therefore, it may be considered that doxapram is classified as a SKF-525A-like agent, which has a biphasic effect of inhibition and induction on the drug metabolism (26).

The hypothermic action of aminopyrine, the muscle relaxative action of meprobamate, the analgesic actions of aminopyrine and morphine, and the lethality of strychnine and picrotoxin were potentiated by the pretreatment with doxapram 60 min before. On the other hand, the antitumor activity of cyclophosphamide on Ehrlich solid tumors was inhibited by the pretreatment with doxapram 60 min before. The above results indicate that doxapram inhibited the metabolism of combined drugs when administered 60 min before these drugs.

It has been well known that aminopyrine is metabolized to 4-methylaminoantipyrine and then to 4-aminoantipyrine which would be biologically inactive (16) and that cyclophosphamide, an alkylating agent, is inactive per se, but is activated by the hepatic microsomal enzyme (27, 28). The potentiating effect of doxapram on the action of aminopyrine and the inhibitory effect on cyclophosphamide were further proven by determination of the plasma levels of these compounds. The concentration of aminopyrine in plasma was elevated by doxapram pretreatment and the rate of decay of aminopyrine in plasma was less in the doxapram-treated group than that in the control group. In addition, the plasma level of normustard determined as an active metabolite of cyclophosphamide in the doxapram-treated group was remarkably lower than that in the control group.

As regards to the hepatic drug-metabolizing system, after the treatment by doxapram, marked decreases in the activities of aminopyrine N-demethylase, aniline hydroxylase, and pentobarbital oxidase were observed; and the level of cytochrome P-450 and the activity of NADPH-cytochrome c reductase slightly decreased. Additionally, a small decrease in the level of cytochrome b₅ and the activity of NADH-ferricyanide reductase were observed with the doxapram treatment. On the other hand, the consecutive administration of doxapram for 7 days raised the activities of aminopyrine N-demethylase, aniline hydroxylase, and NADPH-cytochrome c reductase and the level of cytochrome P-450. It is evident that doxapram affects the hepatic drug-metabolizing system as mentioned above.

Further studies are in progress with the aim of accurately characterizing the mechanism of action of doxapram on hepatic microsomes.

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