Abstract—The effect of lipopolysaccharide (obtained from *Escherichia coli*, LPS) on the antitumor activity, acute toxicity and metabolism of tegafur was investigated in mice in comparison with 5-fluorouracil (5-FU). It was found that the intravenous administration of LPS (1.25 or 2.5 mg/kg) 24 hr prior to tegafur decreased the antitumor activity of tegafur against the solid form of Sarcoma 180. On the acute toxicity of tegafur or 5-FU, the lethality of the former was decreased and that of the latter was enhanced by the pretreatment with LPS 24 hr before. In LPS-treated mice, after the administration of tegafur, the level of tegafur in plasma was higher and the elevated level maintained longer than in untreated mice; and a small amount of 5-FU was released. A high level of 5-FU in plasma after the administration of tegafur or 5-FU was higher, and its conversion of 5-FU to fluorouridine (FUR) was lower than that of control mice. On the other hand, LPS inhibited significantly the hepatic drug-metabolizing enzymes 24 hr after. It can, therefore, be presumed that the antitumor activity of tegafur was affected with LPS as a result of inhibition of conversion from tegafur to 5-FU or from 5-FU to FUR mainly according to depression in the hepatic drug-metabolism.

Tegafur (1-(2'-tetrahydrofuryl)-5-fluorouracil), a masked compound for converting to 5-fluorouracil (5-FU) in vivo, has been widely used as an antitumor agent; and its antitumor activity is very similar to that of 5-FU, but its toxicity is considerably lower (1, 2). In in vitro studies, Toide et al. (3) demonstrated that the conversion of tegafur to 5-FU occurred mainly in the microsomal fraction of the rat liver in the presence of NADPH.

Recently, Sasaki et al. (4) found that the antitumor activity of cyclophosphamide as a masked compound on Ehrlich solid tumor in mice was decreased by the intravenous injection of lipopolysaccharide (obtained from *Escherichia coli*, LPS) or *Mycobacterium butyricum* (MB); and in LPS- or MB-treated mice, the analgesic action of aminopyrine was strengthened and that of codeine was weakened (4). These findings suggest that immunopotentiators inhibited the hepatic microsomal drug-metabolizing system.

The objective of the present study is to elucidate how LPS inhibits the activation of tegafur as a masked compound and the metabolism of 5-FU in vivo. The authors investigated the effect of LPS on the antitumor
activities of tegafur and 5-FU, on the level of these drugs in plasma, and on the activity of hepatic microsomal drug-metabolizing enzymes in mice.

MATERIALS AND METHODS

Animals: Male ddY mice weighing 20–22 g, supplied from the Animal Center of Shizuoka Experiments, were used in the present study. Food and water were provided ad libitum.

Acute toxicity: Tegafur or 5-FU was injected 24 hr after the injection of LPS. Lethality of mice was observed continuously for 16 days.

Tumor cells: Sarcoma 180 ascites tumor cells that had been maintained in ddY mice by weekly passage in our laboratory were used.

Chemotherapy: Solid-type Sarcoma 180 was obtained by the intramuscular injection of ascites cells (1×10^6 cells) into the right thigh of mice. Tegafur or 5-FU was administered once intraperitoneally 24 hr after tumor transplantation. LPS was administered intravenously 6, 12 or 24 hr prior to the administration of antitumor drugs. On day 15, mice were sacrificed, and the tumors were removed and weighed. The antitumor activity was evaluated by the mean weight of tumors in treated mice as a percentage of their mean weight in control mice.

Assays of tegafur and 5-FU in plasma, liver and kidneys: Samples were adjusted to pH 2.0 with 1 N HCl and extracted with 10 volumes of chloroform. Tegafur extracting in the chloroform layer and 5-FU remaining in the aqueous layer were assayed by the method of Fujita et al. (5). The ratio of 5-FU and fluorouridine (FUR) in the tissue was determined by the method of bioassay after separation of active metabolite using paper chromatography (6, 7).

Preparation of enzyme solution and assay: The livers of mice were homogenized in 4 volumes of 1.15% KCl-10 mM sodium potassium phosphate buffer (pH 7.4), and the homogenates were centrifuged at 9,000×g for 20 min to remove cell debris and nuclei. The microsomal fraction of mouse liver was prepared by centrifugation at 105,000×g for 60 min. Microsomal cytochrome P-450 and cytochrome b5 contents were measured according to the method of Omura and Sato (8) with a spectrophotometer (UV 300 type, Shimadzu). The activities of aminopyrine N-demethylase and aniline hydroxylase in the hepatic 9,000×g supernatant fraction were assayed by the method of Kitagawa et al. (9). All subsequent procedures were carried out at 4°C.

Protein assay: Protein concentrations were determined by the method of Lowry et al. (10) using bovine serum albumin as a standard.

Drug used: The drugs were tegafur (1-(2'-tetrahydrofuryl)-5-fluorouracil, Taiho Yakuhin Kogyo Co.), 5-fluorouracil (5-FU, Kyowa Hakko Kogyo Co.), and lipopolysaccharide (obtained from Escherichia coli, Difco Co., LPS). Drugs were dissolved with saline just before use.

Statistical analysis: The Student's t-test was used for statistical analysis. Significance levels of P<0.05 and P<0.01 were used as the criteria of significance.

RESULTS

Effect of LPS treatment on the antitumor activities of tegafur and 5-FU: Effect of LPS treatment on the antitumor activities of tegafur and 5-FU against the solid form of Sarcoma 180 in mice was examined. Figure 1 shows the relationship between the pretreatment time of LPS and the antitumor effect of tegafur. The solid tumor weight (mean±S.E.) in control mice on the 15th day after the implantation of tumor cells was 5.23±0.52 g. When tegafur (400 mg/kg) was once injected intraperitoneally 24 hr after the cell inoculation, the solid tumor weight (mean±S.E.)
Fig. 1. Relationship between the pretreatment time of LPS and the antitumor activity of tegafur on the growth of Sarcoma 180 solid tumor in mice. Groups of ten ddY mice were used. The antitumor activity was estimated from the mean weight of the solid tumor compared with that of the saline-treated mice on the 15th day after Sarcoma 180 cell (1×10^6 cells) inoculation. The cells were inoculated into the left thigh. Tegafur (400 mg/kg) was injected once intraperitoneally 24 hr after the cell inoculation. LPS was injected once intravenously at 0, 12 or 18 hr after the cell inoculation (24, 12 or 6 hr prior to tegafur). □: tegafur alone (control), ■: LPS alone (1.25 mg/kg), ■■: LPS (1.25 mg/kg) plus tegafur, ■■■: LPS alone (2.5 mg/kg), ■■■■: LPS (2.5 mg/kg) plus tegafur. Significant differences from the control value are indicated as *(P<0.05).

Fig. 2. Effect of LPS on the lethality of tegafur and 5-FU. Groups of ten mice were used. Tegafur or 5-FU was injected intraperitoneally 24 hr after the injection of LPS (2.5 mg/kg, i.v.). Lethality of mice was observed continuously for 16 days.

Effect of pretreatment with LPS on the antitumor activity of tegafur: The pretreatment time of LPS (0, 6, 12, and 24 hr prior to tegafur) was found to affect the antitumor activity of tegafur. The pretreatment of 6 hr prior to tegafur significantly decreased the antitumor activity of tegafur. The pretreatment of LPS 24 hr before increased the lethality of 5-FU, particularly at a dose of 200 or 400 mg/kg, i.p., of 5-FU. In contrast, lethality in a group receiving 1,200 mg/kg, i.p., of tegafur decreased significantly by the pretreatment with LPS 24 hr before.

Effect of LPS treatment on the levels of tegafur and 5-FU in plasma, liver and kidneys: Figure 3 shows time courses of the levels of tegafur and 5-FU metabolized from tegafur in plasma of mice after the administration of tegafur (400 mg/kg, i.p.). The level of tegafur in plasma was elevated by the injection of LPS (1.25 or 2.5 mg/kg, i.v.). The level of tegafur in plasma 30 min after the injection in control mice was 543.3±17.8 μg/ml, and

Effect of LPS treatment on the levels of tegafur and 5-FU in plasma, liver and kidneys: Figure 3 shows time courses of the levels of tegafur and 5-FU metabolized from tegafur in plasma of mice after the administration of tegafur (400 mg/kg, i.p.). The level of tegafur in plasma was elevated by the injection of LPS (1.25 or 2.5 mg/kg, i.v.). The level of tegafur in plasma 30 min after the injection in control mice was 543.3±17.8 μg/ml, and
Fig. 3. Effect of LPS on the level of tegafur and 5-FU in plasma of mice after the administration of tegafur. Tegafur was given at a dose of 400 mg/kg (i.p.) 24 hr after the i.v. injection of LPS. Each point represents the mean±S.E. of five values obtained from pooled mixture of the plasma of three mice. ○: saline (control), ●: LPS (1.25 mg/kg), ▲: LPS (2.5 mg/kg). Significant differences from the control value are indicated as *P<0.05 and **P<0.01.

then it gradually decreased, being undetectable at 8 hr after. The rate of decay of tegafur in the plasma of LPS-treated mice may be less than that of control mice at each measured time.

As regards to 5-FU, the level of 5-FU in plasma metabolized from tegafur reached a maximum in 30 min and then rapidly decreased. On the contrary, the level of 5-FU liberated from tegafur in plasma was lower in LPS-treated mice.

On the other hand, after the administration of 5-FU (50 mg/kg, i.p.), the level of 5-FU in plasma was determined at each time expressed in Fig. 4. The degradation of 5-FU was also influenced by LPS (1.25 or 2.5 mg/kg). The level of 5-FU in the plasma of LPS-treated mice was higher than that of control mice.

In addition, the level of tegafur or 5-FU and the ratios of FUR against 5-FU in the liver and kidneys after the administration of tegafur or 5-FU were examined, and the results are shown in Table 1. In LPS-treated mice, the levels of tegafur and 5-FU in the liver and kidneys 30 min after tegafur (400 mg/kg, i.p.) were higher than that in control mice, and the level of 5-FU in the liver and kidneys 20 min after 5-FU (50 mg/kg, i.p.) was higher than that in control mice. The ratios of FUR against 5-FU in the liver and kidneys 30 min after the administration of tegafur or 20 min after the administration of 5-FU were markedly decreased in LPS-treated mice. It means that the conversion of 5-FU to FUR in the liver and kidneys was inhibited by the treatment of LPS. In plasma, FUR could not be detected by the method employed in this paper.

Effect of LPS on the hepatic drug-metabolizing system: The effect of LPS on drug-metabolizing enzymes and on cytochrome P-450 and cytochrome b5 contents was examined in the hepatic microsomal fraction of mice as shown in Fig. 5. Both activities of aminopyrine N-demethylase and aniline hydroxylase decreased 24 hr after the injection of LPS (1.25 or 2.5 mg/kg, i.v.). In the decreases of the activities of drug-metabolizing enzymes in liver microsome by
Table 1. Effect of LPS on the level of tegafur or 5-FU and the ratio of 5-FU and FUR in liver and kidneys of mice after the administration of tegafur or 5-FU of tegafur or 5-FU

| Treatment | Level (µg/g) | Ratio |
|-----------|-------------|-------|
|           | Tegafur | 5-FU : FUR | Tegafur | 5-FU : FUR |
| Control   | Liver | 167.4±15.5 | 1:1.47 | 254.3±23.7 | 1:0.69 |
|           | Kidneys | 184.8±15.3 | 1:1.76 | 283.0±24.8 | 1:0.65 |
| 400 mg/Kg | Liver | 2.80±0.27  | 1:0.78 | 3.85±0.38  |       |
|           | Kidneys | 8.33±0.72  | 1:0.67 | 18.25±1.61 | 1:0.29 |

Tegafur or 5-FU was injected intraperitoneally 24 hr after the injection of LPS (2.5 mg/kg, i.v.). Mice were sacrificed 30 min after tegafur and 20 min after 5-FU. Each value represents the mean of five experiments obtained from the pooled mixture of tissues from three mice. Significant differences from the control value are indicated as **P<0.05 and ***P<0.01.
Fig. 5. Effect of LPS on the activity of aminopyrine N-demethylase and aniline hydroxylase and cytochrome P-450 and cytochrome b5 contents in the hepatic microsomal fraction of mice. Control mice were injected with 0.1 ml/10 g of saline (i.v.). Mice were sacrificed 24 hr after the administration of LPS. Each column is the mean activity and horizontal bars represent the standard errors of the means of 6 mice. Control values were as follows: aminopyrine N-demethylase, 1.86±0.048 (nmol/min/mg of protein); aniline hydroxylase, 1.82±0.061 (nmol/min/mg of protein); cytochrome P-450, 1.01±0.052 (nmol/mg of protein); cytochrome b5, 0.37±0.026 (nmol/mg of protein). LPS, the greatest inhibition was observed with aniline hydroxylase. Similarly, the levels of cytochrome P-450 and cytochrome b5 were also significantly decreased by pretreatment with LPS 24 hr before the test.

DISCUSSION

Metabolism of chemotherapeutic agents in vivo is one of important factors which control the efficacy as well as toxicity of the agents. Recently, a variety of immunopotentiators including bacteria, polysaccharides and low-molecular chemicals have been used clinically for cancer therapy in combination with cancer chemotherapeutic agents. However, there have been a few reports on the influence of immunopotentiators on drug-metabolizing enzymes which mediate the metabolism of chemotherapeutic agents (11–13). Higuchi et al. (14, 15) suggested the effect of lipopolysaccharide on the metabolism of 6-mercaptopurine in mice. From this point of view, the effect of LPS, an immunopotentiator obtained from E. coli, on the actions of tegafur and 5-FU was examined.

Previously, Sasaki et al. (4) found that the antitumor activity of cyclophosphamide on Ehrlich solid tumor was reduced by the intravenous injection of immunopotentiators such as LPS, MB or OK-432, a Streptococcus preparation and that the plasma concentration of normustard relating to an active metabolite of cyclophosphamide after LPS was lower than that after the administration of cyclophosphamide alone. They also found that these immunopotentiators potentiated the actions of pentobarbital and aminopyrine and weakened the action of codeine phosphate (4).

It was known that 5-FU is converted to FUR and subsequently to 5-fluorouridine monophosphate; the latter changes into 5-fluorodeoxyuridine 5'-monophosphate (FdUMP) which is a potent inhibitor of thymidine synthetase (16), while 5-FU is also degraded and excreted in urine as 2-fluoro-3-ureidopropionic acid or 2-fluoro-β-alanine (17).

In the present work, we demonstrated the effect of LPS which is one of the immunopotentiators, on the antitumor activities and plasma concentrations of tegafur and 5-FU and the hepatic drug-metabolizing system in mice.

It was apparent that the effect of LPS on the antitumor activity of tegafur against mice bearing Sarcoma 180 tumor is dependent on the time intervals of pretreatment with LPS. A single administration of LPS (1.25 or 2.5 mg/kg, i.v.) slightly suppressed the growth of Sarcoma 180 solid tumor. Antitumor activity of a single administration of tegafur (400 mg/kg, i.p.) was significantly inhibited by the pretreatment with LPS 24 hr before. In other experiment using Ehrlich ascites tumor, we did not obtain obvious results of the effect of LPS on tegafur action. On the other
hand, the activity of a single administration of 5-FU (50 mg/kg, i.p.) was increased by the pretreatment with LPS 24 hr before, but this was not significant. Sasaki et al. (4) reported in a previous paper that the maximal prolongation of sleeping time induced by pentobarbital was observed 24 hr after the injection of LPS. It is thought from the above result that the maximal inhibition of drug-metabolism induced by LPS occurred 24 hr after the administration of LPS.

From the present toxicity studies of tegafur and 5-FU, the combination of tegafur or 5-FU with LPS resulted in a marked variation of the toxicity. On the 16th day observation after the administration of a large dose of 5-FU in mice, it became apparent that the administration of LPS 24 hr prior to 5-FU enhanced the toxicity of 5-FU. In contrast to 5-FU, the lethality of tegafur was reduced by the administration of LPS 24 hr prior to tegafur. These results indicate that LPS inhibits the conversion from tegafur to 5-FU and the metabolism of 5-FU; and LPS decreases the antitumor activity of tegafur and increases the lethality of 5-FU.

In further studies on the metabolism of tegafur, we showed the time course of the plasma level of tegafur or 5-FU in mice after the administration of tegafur. The activation of tegafur was considerably affected by the pretreatment of LPS. The level of 5-FU released from tegafur in plasma was maximum at 30 min after the administration of tegafur. In LPS-treated mice, a higher concentration of tegafur and a lower concentration of 5-FU in plasma after tegafur were observed as compared with those in mice treated with tegafur alone, while the level of 5-FU in plasma after the administration of 5-FU was entirely opposite in the case of tegafur, that is to say, the level of 5-FU in plasma after 5-FU was a higher level in LPS-treated mice. In the liver and kidneys, the level of 5-FU after tegafur or 5-FU was higher in LPS-treated mice; and LPS depressed the formation of FUR from 5-FU. These facts indicated that LPS inhibited the activation of tegafur and the degradation and the activation of 5-FU. From this point of view, it seems also likely that LPS decreases the antitumor activity of tegafur by inhibiting the conversion from tegafur to 5-FU and from 5-FU to FUR. However, although it was known that 5-FU was converted to FdUMP to become an active metabolite (16), the effect of LPS upon the intricate mechanism responsible for the metabolism of 5-FU is still not clear.

We have examined the influence of LPS treatment on the activity of the hepatic drug-metabolizing enzymes in mice. LPS markedly reduced the activities of aminopyrine N-demethylase and aniline hydroxylase in the 9,000 x g supernatant of the liver homogenate and reduced cytochrome P-450 or cytochrome b5 contents in the microsomes of the liver 24 hr after the injection. Decrease of enzyme activity induced by LPS is probably a consequence of damage to some component of the electron-transport system such as cytochrome P-450 (18, 19). A dose of 2.5 mg/kg, i.v., of LPS corresponds to about one twelfth the LD50 in mice. Furthermore, the depression induced by LPS on the hepatic drug-metabolism did not seem to be related to the secondary occurring phenomenon as a result of the endotoxin syndrome (20, 21). The results indicated that LPS depressed a wide range of reactions in the hepatic drug-metabolizing system; hence, it may be possible to explain the inhibitory effect of LPS on the antitumor activity of tegafur.

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