TISSUE DISTRIBUTION AND BIOTRANSFORMATION OF POTASSIUM OXONATE AFTER ORAL ADMINISTRATION OF A NOVEL ANTITUMOR AGENT (DRUG COMBINATION OF TEGAFUR, 5-CHLORO-2,4-DIHYDROXYPYRIDINE, AND POTASSIUM OXONATE) TO RATS

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ABSTRACT:

S-1, a new oral 5-fluorouracil (5-FU)-derivative antitumor agent, is composed of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate (Oxo). Oxo, which inhibits the phosphorylation of 5-FU, is added to reduce the gastrointestinal (GI) toxicity of the agent. In this study, we investigated the tissue distribution and the metabolic fate of Oxo in rats after oral administration of S-1. Oxo was mainly distributed to the intracellular sites of the small intestines in a much higher concentration than 5-FU, but little distributed to other tissues, including tumorous ones in which 5-FU was observed after oral administration of S-1. Plasma concentration-time profiles of Oxo and its metabolites after i.v. and oral administration of S-1 revealed that Oxo was mainly converted to cyanuric acid in the GI tract. Furthermore, the analysis of drug-related radioactivity in GI contents and in vitro studies suggested that Oxo was converted to cyanuric acid by two routes, the first being direct conversion by the gut flora in the cecum, and the second, conversion by xanthine oxidase or perhaps by aldehyde oxidase after degradation to 5-azauracil (5-AZU) by the gastric acid. These results indicate that, although a part of the administered Oxo was degraded in the GI tract, Oxo was mainly distributed to the intracellular sites of the small intestines in a much higher concentration than 5-FU and that little was distributed to other tissues, including tumors. We conclude that this is the reason why Oxo suppresses the GI toxicity of 5-FU without affecting its antitumor activity.

S-1, a new antitumor agent, was developed based on biochemical modification of 5-fluorouracil (5-FU), and consists of 1-(2-tetrahydrofuryl)-5-fluorouracil, (tegafur; FT), 5-chloro-2,4-dihydroxypridine (CDHP), and monopotassium 1,2,3,4-tetrahydro-2,4-dioxo-1,3,5-triazine-6-carboxylate (potassium oxonate; Oxo) in a molar ratio of 1:0.4:1 (see Fig. 1). FT, which is a prodrug of 5-FU, plays a role as an effector. Both CDHP and Oxo, which do not have antitumor activity themselves, act as modulators. CDHP competitively inhibits dihydroxypyridine dehydrogenase (EC 1.3.1.2), which degrades 5-FU, about 180 times more effectively than uracil does in vitro (Tatsumi et al., 1987), leading to the prolonged retention of an effective concentration of 5-FU in the blood (Uchida et al., 1995). Oxo competitively inhibits pyrimidine phosphoribosyltransferase (EC 2.4.2.10), which converts 5-FU to fluorouridine monophosphate, and leads to relief of the antitumor activity of 5-FU. As to the biological fate of Oxo, the metabolites have been identified as 5-azauracil (5-AZU) (Shirasaka et al., 1993) and cyanuric acid (CA) (Yamamoto et al., 1997), which have no pyrimidine phosphoribosyltransferase inhibitory activity, however, their metabolic pathways are unknown. In this study we examined the tissue distribution of Oxo and compared the concentration of Oxo with that of 5-FU in the small intestines and tumor tissue after oral administration of S-1. In addition we investigated the details of the metabolic pathway of Oxo.

Materials and Methods

An important cytostatic effect of 5-FU is mediated through formation of the phosphorylated metabolite 5-fluoro-2'-deoxyuridylate, which together with methylenetetrahydrofolic acid binds to thymidylate synthetase (EC 2.1.1.45) and forms a stable ternary complex, resulting in significant enzyme inhibition (Langebach et al., 1972; Santi and McHenry, 1982). Formation of 5-fluoro-2-uridinetriphosphate results in incorporation of the drug into RNA, and many studies have suggested the importance of incorporation of 5-FU into RNA or DNA as correlates of cytotoxicity (Carriço and Glazer, 1979). GI toxicity of 5-FU is also caused by its phosphorylation in the GI tract (Houghton et al., 1979). The reduction in GI toxicity of 5-FU after administration of S-1 is related to the concentrations of Oxo and 5-FU in the small intestine, whereas the distribution of Oxo to tumors might decrease the antitumor activity of 5-FU. As to the biological fate of Oxo, the metabolites have been identified as 5-azauracil (5-AZU) (Shirasaka et al., 1993) and cyanuric acid (CA) (Yamamoto et al., 1997), which have no pyrimidine phosphoribosyltransferase inhibitory activity.

Abbreviations used are: 5-FU, 5-fluorouracil; Oxo, potassium oxonate; GI, gastrointestinal; CA, cyanuric acid; 5-AZU, 5-azauracil; FT, 1-(2-tetrahydrofuryl)-5-fluorouracil; CDHP, 5-chloro-2,4-dihydroxypyridine; PEG, polyethylene glycol; S9, 9000g supernatant fraction; SKF-525A, α,β-dihydroximinoethyl diphenylpropylacetate.
μCi/mg, was synthesized by Daiichi Pure Chemicals Co., Ltd. The radiochemical purity of the [14C]Oxo was more than 98%. The structures of FT, CDHP, and Oxo and the positions labeled in [14C]Oxo are shown in Fig. 1. Polyethylene glycol 4000, 1,2-3H-labeled ([3H]PEG; 59.5 MBq/g), was purchased from Daiichi Pure Chemicals Co., Ltd. (Ibaraki, Japan). Xanthine oxidase from buttermilk and CA were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Allopurinol and chloropromazine hydrochloride were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and SKF-525A came from Paesel+Lorei (Frankfurt, Germany). All reagents and solvents were commercially available and guaranteed to be reagent grade or HPLC grade.

**Preparation of Test Samples.** Because the active component in S-1 is FT, the dosage was indicated as FT dose. S-1 for oral administration was dissolved at 5 mg/5 ml in 0.5% (w/v) hydroxypropylmethylcellulose solution and for i.v. administration, at 5 mg/ml in 25 mM sodium hydrogen carbonate solution. When [14C]Oxo was administered orally as S-1, the radioactive dose was 20.65 MBq/kg, and when [14C]Oxo was coadministered orally as S-1 with [3H]PEG, the radioactive doses of [14C] and [3H] were 1.44 and 0.52 MBq/kg, respectively.

**Animals and Tumors.** Donryu strain male SPF rats (6 weeks old) were purchased from Nihon SLC Co., Ltd. (Shizuoka, Japan) and had free access to tap water and commercially available chow (CE-2, Clea Japan Inc., Tokyo, Japan). The rats were fasted overnight before experiments and for 8 h after dosing, but were supplied with water ad libitum. Yoshida sarcoma was prepared by s.c. transplantation of 1 × 10⁵ cells taken from the back of Donryu rats to prepare the tumor-bearing rats, and the experiments were performed on nonfasting animals when the tumor had reached almost 1 g in size.

**Tissue Distribution Studies.** For the GI distribution study of drug-related radioactivity, rats were orally coadministered 5 mg/kg S-1 containing [14C]Oxo with 8.8 mg/kg [3H]PEG as a nonabsorbable marker. Stomach, small intestines, and large intestines were removed at 1, 2, 4, and 6 h after the administration. The rats were fasted overnight before experiments and for 8 h after dosing, but were supplied with water ad libitum. Yoshida sarcoma was prepared by s.c. transplantation of 1 × 10⁵ cells taken from the back of Donryu rats to prepare the tumor-bearing rats, and the experiments were performed on nonfasting animals when the tumor had reached almost 1 g in size.

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**Collection of Gastric Juice and Preparation of Cecum Content Homogenate, Small Intestinal S9, and Hepatic S9.** Rats were sacrificed for collection of gastric juice and for preparation of cecum content homogenate and 900g supernatant fraction (S9) of the small intestines and liver. For collection of the gastric juice, rats were laparotomized under anesthesia, and their stomachs were clamped at the fundus ventriculi and cannulated with a polyethylene cannula from the pyloric valve. Tetrastigmine at 1.5 μg/kg was infused through a lateral tail vein, and naturally flowing gastric juice was collected via the cannula, pooled, and then measured for pH value. Aliquots of the collected gastric juice were neutralized by sodium hydroxide solution. Cecum content homogenate was prepared by homogenizing the cecal contents with water at a final concentration 60% (w/v), and aliquots of the homogenate were sterilized by autoclaving (125°C, 25 min). S9 of the small intestine and liver was prepared essentially according to previously reported methods (Silva and Hatfield, 1978). All prepared samples were stored frozen at −20°C or −80°C until used.

**In Vitro Studies.** To study the degradation of Oxo, we added a final concentration of 100 μM [14C]Oxo to gastric juice, neutralized gastric juice, cecum content homogenate, or sterilized cecum content at the start of incubation. In the case of S9 of the small intestines or liver, Oxo and cofactor solution were added. The composition of the cofactor solution was phosphoribosyl pyrophosphate (20 mM), magnesium chloride (50 mM), and 20 mM potassium phosphate buffer (pH 6.8). The reaction was initiated by the addition of cold acetone, after neutralization by sodium hydroxide solution in the case of incubation with gastric juice, and extracted samples were centrifuged at 3000 rpm, 4°C for 15 min in a Himac CF7D2 centrifuge (Hitachi, Tokyo, Japan). Aliquots of the supernatant were subjected to HPLC analysis to determine the radioactive metabolites. To study the in vitro metabolism of 5-AZU, we performed incubation for 20 min at 37°C in 50 mM Tris-HCl (pH 7.4) in glass tubes. The reaction was initiated by the addition of 5-AZU after a 1-min preincubation with hepatic S9 or purified xanthine oxidase with or without inhibitor (1 mM allopurinol, 1 mM SKF-525A, or 1 mM chloropromazine), and stopped by the addition of cold acetone. CO-treated hepatic S9, which was bubbled with CO gas for 1 min just before the reaction, was also incubated with 5-AZU, and the reaction was stopped in the same manner. Deproteinized samples were centrifuged, and aliquots of the supernatant were subjected to HPLC to determine the metabolites of 5-AZU.

**Analysis.** Concentrations of Oxo and 5-FU in plasma and analysis samples prepared from tumors and small intestines after administration of S-1 were determined as previously described (Matsushima et al., 1997). Oxo was cleaned up with an anion exchange column (Bond Elut NH2), and the extracted Oxo was degraded to 5-AZU and converted to pentafluorobenzyl derivatives. 5-FU was extracted with ethyl acetate from samples and converted to pentafluorobenzyl derivatives. These derivatives were analyzed by gas chromatography-negative ion chemical ionization mass spectrometry using [15N₁₂]Oxo and [15N₂]FU as an internal standard.

The plasma concentration of CA was determined as follows: To 0.5 ml of the plasma, 0.05 ml of the internal standard solution ([15N₂]-CA: 1 μg/ml) and 0.5 ml of Tris buffer were added, and mixed. This mixture was applied to an AG1–8X column (Bio-Rad, Hercules, CA), and the column was then washed with 4 ml of water and 2 ml of 1% acetic acid and subsequently eluted with 4 ml of 1% acetic acid. The eluant was evaporated to dryness with a SpeedVac Concentrator (Savant, Farmingdale, NY), and 0.03 ml of acetonitrile, 0.01 ml of benzylbromide, and 0.01 ml of triethylamine were added to the residue. The
the radiolabel reached the large intestines from there. The radioactivity related to $^{14}\text{C}$Oxo in the stomach was less than that in the small and large intestines.

**Distribution of Oxo and 5-FU.** Table 1 shows the mean concentrations of Oxo in selected tissues and 5-FU in the small intestines and tumor for groups of animals ($n = 3$) at 1 and 6 h after the oral administration of S-1 to tumor-bearing rats. Oxo was distributed at a higher concentration to GI tissues than to the plasma but was poorly distributed to liver, kidney, spleen, lung, and bone marrow. Compared with 5-FU in the small intestines and tumor, Oxo was found at a much higher concentration in the small intestines but was not detected in tumor tissue, in which 5-FU was observed. On the other hand, CA was detected in all selected tissues, and 5-AZU was never detected (data not shown).

**Plasma Concentration-Time Profiles of Oxo and Its Metabolites after i.v. and p.o. Administration of S-1.** Figure 3 shows the averaged plasma concentration-time profiles of Oxo and its metabolite, CA, in rats after i.v. or p.o. administration of S-1. In plasma 5-AZU was undetectable after administration by either route. The plasma concentration of CA after i.v. administration of S-1 was markedly less than that observed after the oral administration. Table 2 shows the AUC$_{(0-t)}$ of Oxo and CA after i.v. or oral administration of S-1 to rats. After oral administration, the AUC$_{(0-t)}$ of CA was 58 times greater, and that of Oxo was decreased compared with that observed after the oral administration.

**Composition of Drug-Related Radioactivity in GI Contents.** The composition of drug-related radioactivity in GI contents for the three groups of tumor-bearing rats at 1 and 6 h after oral administration of $^{14}\text{C}$Oxo as S-1 is shown in Table 3. Although Oxo was found to a greater extent in gastric and small intestinal contents than CA, the amount of CA was greater than that of Oxo in the cecal contents and almost the same as that of Oxo in the large intestines at 6 h. On the other hand, 5-AZU was detected in the gastric contents only.

**In Vitro Degradation of Oxo.** Table 4 shows the percentage of Oxo remaining and that of 5-AZU, CA, and others generated after the GI distribution profiles of drug-related radioactivity in stomach, small intestines, and large intestines after oral coadministration of $^{[3]}\text{H}$PEG (8.8 mg/kg) and S-1 (5 mg/kg) containing $^{[14]}\text{C}$Oxo to rats.

Values are shown as the mean of three animals.
incubation with gastric juice, cecum content homogenate, and S9 of small intestines or liver. Oxo was degraded to 5-AZU in gastric juice having a pH value of 1.6, but its degradation did not occur in neutralized gastric juice (pH 7.0). Also, Oxo was converted to CA in the cecum content homogenate, but its conversion was decreased by sterilization of the homogenate. In hepatic S9, Oxo was converted to five unknown metabolites (others) after incubation with the cofactor phosphoribosyl pyrophosphate, but was stable in small intestinal S9.

**Biotransformation of 5-AZU to CA in Vitro.** Table 5 shows the changes of conversion of 5-AZU to CA in hepatic S9 or purified xanthine oxidase after incubation with inhibitor or pretreatment of hepatic S9 by CO bubbling. 5-AZU was converted to CA after incubation with hepatic S9 or purified xanthine oxidase, which was confirmed by comparison with authentic samples, and the amount produced by rat hepatic S9 and purified xanthine oxidase from buttermilk was 10.00 nmol/min/mg of protein and 389.9 nmol/min/IU, respectively. In hepatic S9, incubation with allopurinol, SKF-525A, or chlorpromazine decreased the conversion to CA, and co-addition of allopurinol and SKF-525A inhibited it completely. However, CO bubbling pretreatment of hepatic S9 had no effect on the conversion of 5-AZU to CA. The oxidation activity of xanthine and phthalazine, which are typical substrates for xanthine oxidase and aldehyde oxidase (Johnson et al., 1984), respectively, was decreased by the addition of allopurinol and SKF-525A, respectively (data not shown). Furthermore, in the case of purified xanthine oxidase from buttermilk, incubation with allopurinol completely inhibited the conversion of 5-AZU to CA, but SKF-525A had little effect on it.

**Discussion**

GI distribution profiles of drug-related radioactivity in rats after oral coadministration of [14C]Oxo-containing S-1 and [3H]PEG as a nonabsorbable marker indicated that the radioactivity related to [14C]Oxo was mainly distributed to small intestine, with a maximum concentration achieved at 2 h. On the other hand, the radioactivity associated with [3H]PEG was little distributed to any GI tissue throughout the experimental time period. Furthermore, we investigated the tissue distribution of Oxo and 5-FU at 1 and 6 h after oral administration of S-1 to tumor-bearing rats. Oxo was mainly distributed to GI tissues and was distributed much less to other tissues. Compared with 5-FU in the small intestines and liver, Oxo was distributed to the small intestines at a much higher concentration, but was not detected in the tumor, in which 5-FU was observed. These results suggest that Oxo was distributed to the intracellular sites of the small intestines sufficiently to inhibit the phosphorylation of 5-FU there, because the IC50 value of Oxo for conversion of 1 μM 5-FU to its phosphorylated derivative is 3.7 μM (Shirasaka et al., 1993). We suggest that the different distributions of Oxo and 5-FU to the small intestines and tumor are the reason why Oxo reduces the GI toxicity of 5-FU without affecting its antitumor activity.

**TABLE 3**

<table>
<thead>
<tr>
<th>Material Time after Administration</th>
<th>Oxo</th>
<th>CA</th>
<th>% of dose</th>
<th>5-AZU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach 1 h</td>
<td>1075.3 ± 61.7</td>
<td>42.6 ± 6.9</td>
<td>35.1 ± 8.9</td>
<td></td>
</tr>
<tr>
<td>Contents 6 h</td>
<td>165.8 ± 128.6</td>
<td>7.6 ± 4.4</td>
<td>9.6 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>Small intestinal CO bubbling 1 h</td>
<td>1993.1 ± 119.7</td>
<td>19.0 ± 5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contents 6 h</td>
<td>213.0 ± 70.9</td>
<td>2.8 ± 2.8</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Cecal 1 h</td>
<td>580.0 ± 172.9</td>
<td>800.4 ± 215.0</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Contents 6 h</td>
<td>1217.4 ± 52.6</td>
<td>214.7 ± 51.9</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Values are shown as the mean of three (A) or four (B) animals.
Plasma concentration-time profiles of CA, which was the only metabolite detected in vivo after i.v. and oral administration of S-1, indicated that the concentration after the i.v. administration was markedly lower than that after the oral administration. On the other hand, the oral bioavailability of Oxo was almost 25%. These results suggest that the conversion of Oxo to CA occurred mainly in the GI tract after oral administration. To investigate the metabolic pathway of Oxo to CA, we examined the composition of drug-related radioactivity in GI contents and the in vitro metabolism of Oxo. Although the drug-related radioactivity in the gastric or small intestinal contents was almost associated with Oxo, the amount of radioactivity related to CA was increased in the cecum and large intestinal contents at 6 h after administration of S-1, and the radioactivity related to 5-AZU was detected in the gastric content only. Incubation of Oxo in acidic gastric juice produced 5-AZU, and its production was markedly lower than that after the oral administration. On the other hand, the oral bioavailability of Oxo was almost 25%. These results suggest that the conversion of Oxo to CA occurred mainly in the GI tract after oral administration. To investigate the metabolic pathway of Oxo to CA, we examined the composition of drug-related radioactivity in GI contents and the in vitro metabolism of Oxo. Although the drug-related radioactivity in the gastric or small intestinal contents was almost associated with Oxo, the amount of radioactivity related to CA was increased in the cecum and large intestinal contents at 6 h after administration of S-1, and the radioactivity related to 5-AZU was detected in the gastric content only. Incubation of Oxo in acidic gastric juice produced 5-AZU, and its production was markedly decreased by neutralization of the juice. This finding is in agreement with the results of Lalart et al. (1981), who reported that oxonic acid is rapidly decarboxylated in acidic solution to give 5-AZU (Lalart et al., 1981). In the homogenate of the cecum contents, Oxo was converted to CA, and in hepatic S9 it generated unknown metabolites, which were not detected in vivo, in the presence of phosphoribosyl pyrophosphate, which is a cofactor of orotate phosphoribosyl transferase (Shostak and Jones, 1992). Furthermore, 5-AZU was converted to CA in hepatic S9 and by purified xanthine oxidase, and its conversion was inhibited by the addition of allopurinol, an inhibitor of xanthine oxidase (Massey et al., 1970), and was also decreased by chloropromazine, which was shown to inhibit the activity of aldehyde oxidase (Sugihara et al., 1999; Jordan et al., 1999) and by SKF-525A, an inhibitor of aldehyde oxidase (Robertson and Bland, 1993) and cytochrome P-450 (Testa and Jenner, 1981). However, the conversion was not affected by CO-bubbling pretreatment of hepatic S9 to inhibit cytochrome P-450. These results suggest that Oxo was degraded to 5-AZU by the acidic condition in the stomach and then converted to CA by xanthine oxidase or perhaps by aldehyde oxidase detected in most tissues (Moriwaki et al., 1997, 1996), or Oxo was converted directly to CA by the gut flora in the cecum (Fig. 4).

In conclusion, although a part of Oxo, which is a component in S-1 to reduce the GI toxicity of 5-FU, is degraded in the GI tract after oral administration of S-1, Oxo is distributed to the intracellular sites of the small intestines to a much higher concentration than 5-FU, but was not detected in tumor or other tissues. This difference in distribution between Oxo and 5-FU in the small intestines and tumor is the reason why Oxo suppresses the toxicity of 5-FU in the GI tract without causing a loss of the antitumor activity of 5-FU (Shirasaka et al., 1993). In addition, Oxo is converted to mostly CA in the GI tract by way of two routes. The first route is the direct conversion to CA by gut flora in the cecum, and the second route is degradation to 5-AZU by acid in the stomach and then conversion to CA by xanthine oxidase or aldehyde oxidase.

**References**


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**TABLE 4**

Degradation of [14C]Oxo after a 1-h incubation in gastric juice, cecum content homogenate, small intestinal S9, hepatic S9, neutralized gastric juice, or sterilized cecal content homogenate

Each value represents the means ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Material</th>
<th>Oxo*</th>
<th>5-AZU*</th>
<th>CA*</th>
<th>Others*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric juice (pH 1.6)</td>
<td>27.6</td>
<td>68.4 ± 2.0</td>
<td>ND</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Cecum content homogenate</td>
<td>33.4</td>
<td>58.9 ± 2.8</td>
<td>ND</td>
<td>7.7 ± 1.9</td>
</tr>
<tr>
<td>Small intestinal S9</td>
<td>89.6</td>
<td>5.4 ± 2.0</td>
<td>ND</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>Hepatic S9</td>
<td>6.3</td>
<td>0.8 ± 0.7</td>
<td>4.0 ± 0.9</td>
<td>90.6 ± 0.8</td>
</tr>
<tr>
<td>Neutralized gastric juice (pH 7.0)</td>
<td>97.7</td>
<td>0.2 ± 0.3</td>
<td>ND</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>Sterilized cecal content homogenate</td>
<td>87.3</td>
<td>5.2 ± 0.2</td>
<td>ND</td>
<td>7.5 ± 1.1</td>
</tr>
</tbody>
</table>

ND, not detected.

* Percentage of Oxo after incubation.
* Percentage generated after incubation.
* Percentage of Oxo after incubation.

**TABLE 5**

Metabolism of 5-AZU by hepatic S9 or purified xanthine oxidase from buttermilk and separate effects of allopurinol, SKF-525A, chloropromazine, and CO-bubbling pretreatment of hepatic S9 on its metabolism

Each value represents the means ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Inhibitor</th>
<th>CA Production (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic S9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1 mM allopurinol</td>
<td>10.00 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>+1 mM SKF-525A</td>
<td>8.15 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>+1 mM chloropromazine</td>
<td>4.27 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>+1 mM allopurinol and SKF-525A</td>
<td>5.68 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>CO-treated hepatic S9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme Source</td>
<td>Inhibitor</td>
<td>CA Production (nmol/min/minIU)</td>
</tr>
<tr>
<td>Purified xanthine oxidase</td>
<td>+1 mM allopurinol</td>
<td>389.9 ± 16.1</td>
</tr>
<tr>
<td></td>
<td>+1 mM SKF-525A</td>
<td>320.6 ± 21.2</td>
</tr>
</tbody>
</table>

**FIG. 4.** Proposed metabolic pathway of Oxo after oral administration of S-1.

**KOOC**

\[ \text{stomach} \rightarrow \text{acid in gastric juice} \rightarrow \text{CO}_2 \]

\[ \text{5-AZU} \rightarrow \text{xanthine oxidase or aldehyde oxidase} \rightarrow \text{CA} \]

\[ \text{Oxo} \rightarrow \text{gut flora in cecum} \]


