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CCC: 0377-8282/98

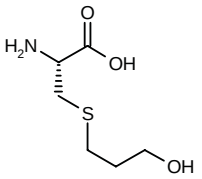
Fudosteine

Expectorant

Prop INN

SS-320A

S-(3-Hydroxypropyl)-L-cysteine
(–)-3-(3-Hydroxypropylsulfanyl)-L-alanine
2(*R*)-Amino-3-(3-hydroxypropylsulfanyl)propionic acid



$C_6H_{13}NO_3S$ Mol wt: 179.23

CAS: 013189-98-5

EN: 170989

Synthesis

Fudosteine has been obtained by two similar ways: Scheme 1.

1) By condensation of L-cysteine (I) with 3-bromopropyl alcohol (II) by means of NaOH in water at room temperature (1, 2).

2) By condensation of L-cysteine (I) with allyl alcohol (III) by means of potassium persulfate in water (3).

Description

Crystals, m.p. 198-202 °C (decomp.), $[\alpha]_D$ –22 ± 2° (c 1.0, water) (room temp.).

Pharmacological Actions

Fudosteine (SS-320A) is a new mucoactive cysteine derivative. An *in vivo* study examined the effects of fudosteine on pathological changes in rat airway epithelium induced by exposure to SO_2 and administration of other drugs. Four different types of goblet cells in the tracheal epithelium were classified according to their size and affinity for Alcian blue (AB) or periodic acid Schiff (PAS) staining. Isoproterenol (0.05 mg/kg/day i.p.) increased the number of goblet cells containing acidic glycoproteins stained with AB and PAS, but had no effect on the number of goblet cells containing neutral glycoprotein stained with PAS only. Fudosteine (100 and 500 mg/kg/day p.o.) inhibited acid glycoprotein-containing goblet cell hyperplasia, and an oral dose of 500 mg/kg/day reduced the elevated number of goblet cells in advanced stage of SO_2-induced bronchitis. The compound also inhibited hypertrophy of lymph follicles, accumulation of airway epithelial cells and infiltration of airway mucosa by inflammatory cells, plasma cells and lymphocytes in bronchitic rats. In rats with reserpine-induced cystic fibrosis, fudosteine did not affect concentrations of total phosphatidylcholine, disaturated phosphatidylcholine, fucose or *N*-acetylneuraminic acid (NANA) in bronchoalveolar lavage fluid. The study demonstrates that fudosteine has no effect on pulmonary surfactant or mucus glycoprotein secretion in rats with cystic fibrosis, but that it does reduce hypersecretion in bronchitic rats (4).

The effects of fudosteine on airway mucus were evaluated in rats and rabbits with bronchitis induced by long-term exposure to SO_2. In rats, an oral dose of fudosteine (500 mg/kg/day) produced a small reduction in total protein and albumin levels in bronchoalveolar lavage fluid, but had no effect on phosphatidylcholine or fucose levels. The compound significantly improved the reduction in free unconjugated NANA. When administered to bronchitic rabbits, fudosteine (500 mg/kg/day) significantly decreased the fucose/NANA ratio in sputa sampled using a modified Perry-and-Boyd method. The drug also demonstrated a tendency to reduce the albumin content in BALF. Thus, it appears that fudosteine alters airway mucus composition in bronchitis and demonstrates mucoregulatory properties (5).

The effects of fudosteine on mucus secretion were evaluated in the human pulmonary mucoepidermoid carcinoma cell line NCI-H292. The cell line secretes hyaluronidase-resistant high-molecular-weight glycoconjugates (HMWG), which were eluted in the void volume on Superose 6HR size-exclusion chromatography. Exposure of these cells to ATP, bradykinin and metha-

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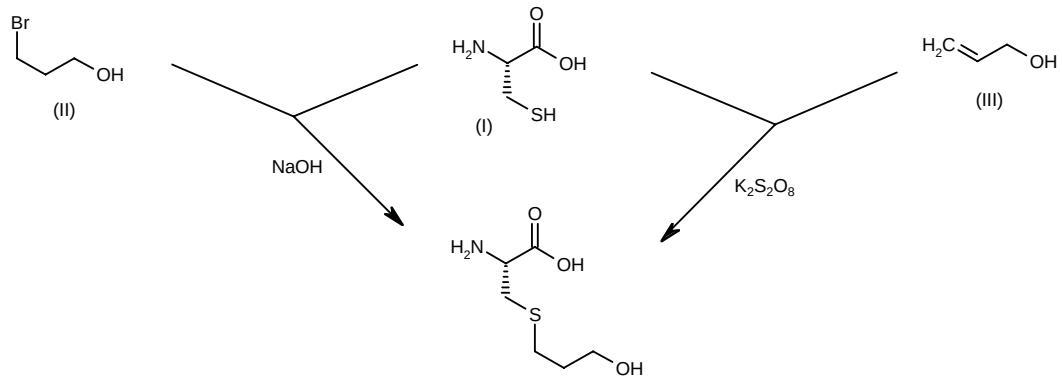
Scheme 1: Synthesis of Fudosteine



choline led to increases in basal secretion of [^{14}C]-glucosamine-labeled HMWGs. Fudosteine, at a concentration corresponding to the effective concentration in plasma from clinical settings (10 μM), significantly reduced HMWG secretion induced by ATP or bradykinin, but had no effect on methacholine-induced HMWG secretion. Other mucoactive drugs such as ethylcysteine, ambroxol and carboxymethylcysteine had no effect on either the ATP- or the bradykinin-induced HMWG secretion in this cell line, suggesting that fudosteine exerts its inhibitory activity on secretion through a different mechanism (6).

Pharmacokinetics and Metabolism

The absorption, metabolism and excretion of a single 25 mg/kg oral dose of [^{14}C]-labeled fudosteine were examined in fasting male dogs. C_{max} in blood was determined to be 23.44 μg/ml at 3.33 h after administration and declined with a $t_{1/2z}$ of 15.8 h, while $AUC_{0-\infty}$ was calculated to be 415.5 μg.h/ml. C_{max} in plasma (35.42 μg/ml) was reached 2 h following administration and declined with a $t_{1/2z}$ of 18.0 h, while the corresponding $AUC_{0-\infty}$ in plasma was 567.3 μg.h/ml. *In vitro* protein binding ratios were 0% in plasma samples from dogs at concentrations of 0.4-40 μg/ml, while protein binding ratios *in vivo* were 0.4-2.5%. *In vitro* distribution ratio of fudosteine in blood cells was determined to be 2.5-9.3%, while *in vivo* values for this parameter in male dogs were 1.0% after 0.5 h, 26.9% after 8 h and 27.2% after 24 h. Major metabolite composition in plasma was as follows: M1, 25.6-37.6%; M4, 7.9-12.3%; and M3, 5.9-12.3% (Fig. 1). In urine, major metabolites and their distribution ratios were: M1, 55.7%; M3, 19.6%; M2, 12.6%; and M4, 5.9%. The parent compound was present in plasma at 32.7-49.9% for a period of 0.5-8 h following administration. Examination of excretion routes demonstrated that 92.9% of the original dose was excreted in the urine and 2.2% was excreted in the feces within a period of 168 h following administration (7).

A similar study evaluated the absorption, distribution, metabolism and excretion of [^{14}C]-labeled fudosteine in male rats during and after administration of 10 doses of 25 mg/kg/day p.o. Blood concentrations of the drug were unchanged by repeated dosing when measured 24 h after each individual administration. However, C_{max} increased by 1.9-fold to a final value of 13.46 μg/ml and $t_{1/2}$ increased by 1.8-fold to a final value of 109.6 h after the last dose as compared to single-dose administration. Tissue concentrations of fudosteine within 0.5 h after the 5th and 10th dose were similar to those after the 1st dose, while concentrations 24 h after the 5th and 10th dose were elevated by repeated dosing. Radioactivity accumulated mainly in adipose and brown adipose tissue. Protein binding ratios in plasma measured at 0.5, 4 and 8 h after the last dose were 9.6, 13.4 and 50.9%, respectively, and radioactivity distribution ratios in blood cells measured at 0.5, 4 and 8 h after administration were 24.7, 28.3 and 32.1%, respectively. Major metabolite tissue distribution and composition 1 h after the 10th dose were as follows: in plasma, mainly parent compound and M4; in urine, main metabolites were M1, M3 and a minor amount of M2; M1 was the main metabolite found in the eyeball and lung, while mainly M1 and M3 were detected in the kidney; M3 was the sole metabolite found in the cerebrum and liver. The excretion of radioactivity in urine and feces reached a maximum level of approximately 96% after the 3rd dose and was maintained at this concentration until the end of the study (8).

A third study evaluated the absorption, distribution, metabolism, excretion and transfer into the fetus and milk of [^{14}C]-labeled fudosteine in male and female rats after a single oral dose of 25 mg/kg. Blood C_{max} (15.03 μg/ml) was reached after 0.31 h in fasting male rats and declined with a $t_{1/2z}$ of 5.7 h, while the $AUC_{0-\infty}$ was calculated to be 33.0 μg.h/ml. Comparison of plasma pharmacokinetic parameters in fasting and nonfasting rats revealed that C_{max} values were lower in nonfasting rats and t_{max} and $t_{1/2z}$

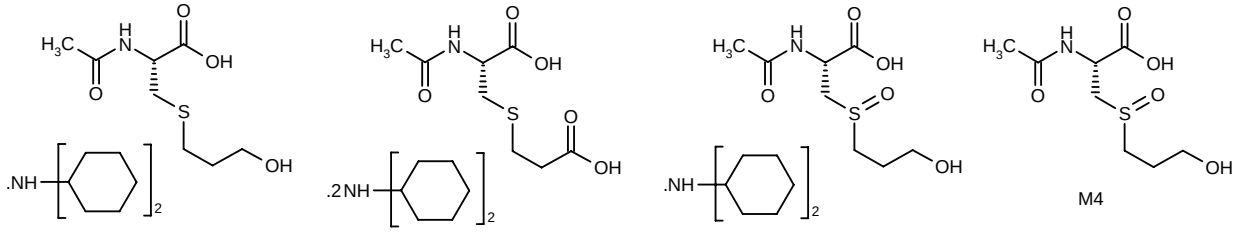


Fig. 1.

values were longer, while AUCs increased 1.3-fold in nonfasting rats as compared to fasting rats. Evaluation of gastrointestinal absorption *in situ* revealed that the majority of the administered dose was absorbed in the small intestine. Maximum concentrations in all tissues evaluated in fasting male rats were reached at 0.5 h, and the drug was eliminated rapidly by 24 h after oral administration, with high concentrations observed in the kidney, liver, cerebrum and eyeball. However, levels of radioactivity in all tissues decreased to less than 1% of maximum values within 120 h after administration. T/P values in most tissues in nonfasting rats were similar, while elimination rates from tissues were lower than in fasting rats. *In vitro* protein binding ratios in plasma samples from rats and humans were low at 0.2-20 μg/ml, and *in vivo* protein binding ratios in rats were 4.0% at 0.5 h, 2.5% at 4 h and 19.5% at 8 h following oral administration. *In vitro* percent distribution of fudosteine-originating radioactivity in blood cells from fasting rats and humans was found to be 28 and 35%, respectively, at concentrations of 0.2-20 μg/ml. The *in vivo* distribution in blood cells from male rats was approximately 25% at 0.5-8 h following oral administration of fudosteine. Evaluation of metabolite distribution showed that M1 and M3 were the primary metabolites found in urine, while parent drug, M1, M3 and M4 were the main constituents in plasma 1 h after oral administration in both fasting and nonfasting rats. In fasting rats, M1 was the main metabolite present in the cerebrum, eyeball and lung, while the parent compound was found mainly in the liver and kidneys. A total of 96.8% of fudosteine was excreted in urine, while 0.7 and 0.8% was excreted in feces and expired air, respectively, 120 h following administration to male rats. Similar results were obtained following intravenous administration, with no apparent gender-based differences observed. Forty-eight hours following oral administration to male rats, 95.4% of fudosteine-originated radioactivity was excreted in urine, while 1.3% was excreted in bile and 0.1% was excreted in feces. Radioactivity concentrations in the fetus exceeded maternal plasma concentrations on the 12th and 18th day of gestation. Fudosteine levels in milk were inferior to plasma levels for the initial 2 h following administration to lactating female rats on the 11th day postdelivery, matched plasma concentrations after 4 h and were superior after 8 h (9).

Toxicity

The results from reproductive and developmental toxicity studies as well as chronic toxicity studies have been reported. In one study, fudosteine was administered daily at doses of 150, 300 and 600 mg/kg by gavage in rabbits from day 6 to 18 of pregnancy. The 600 mg/kg dose induced abortion in late pregnancy in 3 out of 5 dams, while the 300 and 600 mg/kg doses produced erosion in the stomach and/or the duodenum, but had no effects on body weight or food intake. No effects on the viability, teratogenicity, body weight or degree of ossification were observed in fetuses. The general toxic dose in dams was estimated to be 150 mg/kg/day, while the dose producing reproductive toxicity in dams was estimated to be 300 mg/kg/day. The toxic dose for fetal viability and development was determined to be 600 mg/kg/day (10).

A second study evaluated the reproductive and developmental toxicity of fudosteine administered at doses of 125, 500 and 2000 mg/kg/day by gavage to rats on days 7 through 17 of pregnancy. Body weight and food intake decreased temporarily during the treatment period, while general condition, gestation index and nursing conditions were unaffected. Decreases in placental weight of fetuses were observed in the 2000 mg/kg fudosteine group. Adverse effects on fetal viability and teratogenicity were absent in all treatment groups, as were effects on growth, differentiation, reflex function, behavior, learning ability and reproductive performance of the offspring. This particular study estimated the nontoxic dose level to be 500 mg/kg/day for general toxicity in dams, while the reproductive toxicity dose level in dams was estimated to be more than 2000 mg/kg/day. Reproductive toxicity dose levels in fetuses and offspring were estimated to be 500 and 2000 mg/kg/day, respectively (11).

A third reproductive and developmental toxicity study evaluated fudosteine administered at doses of 125, 500 and 2000 mg/kg/day to male rats 9 weeks before and throughout the mating period, while in female rats drug administration was initiated 2 weeks before mating and continued until the 7th day of pregnancy. Parents demonstrated no treatment-related effects on body weight, food consumption, estrus cycle in females, copulation, insemination or fertility indices. Likewise, fudosteine did not affect viability, teratogenicity, body weight or degree of ossification in fetuses. This study indicated that the non-

toxic dose level for general toxicity in males and females, reproductive performance and fetal development was 2000 mg/kg/day in all cases (12).

To evaluate the reversibility of toxicity, fudosteine was administered orally to rats at doses of 0, 100, 300 and 900 mg/kg/day over 12 months, followed by a 3-month drug withdrawal period. Rats were sacrificed after the 12th month of drug treatment and after the withdrawal period. No treatment-related deaths occurred during the course of the study. A solid perineal region in male and female rats receiving 900 mg/kg/day fudosteine was the only clinical sign observed. Males in the 900 mg/kg/day group increased their dietary intake but their body weight did not change, and urinalysis indicated increased urinary volume and sodium excretion. Males in the 300 mg/kg/day group increased their water consumption, and a decrease in urinary pH was observed in males receiving 300 mg/kg or more and in females receiving 900 mg/kg/day. Fudosteine treatment did not induce any observable ophthalmological or hematological changes. Males in the 900 mg/kg/day treatment group demonstrated increased total cholesterol, free cholesterol, triglycerides, free fatty acids and phospholipid levels. Autopsies of the animals revealed no abnormalities apart from solid perineal regions. Liver weight increased in males from the 900 mg/kg/day treatment group, while increased kidney weight was observed in both males and females from the same dose group. Decreased prostate weight was observed in males receiving fudosteine at 100 mg/kg/day or more. Histopathological examinations revealed only a basophilic change in renal tubules in males from the 900 mg/kg/day group, while females demonstrated no treatment-related changes. All changes were reversible within the 3-month drug withdrawal period. The nontoxic dose level of fudosteine administered orally was estimated to be 100 mg/kg/day (13).

Developmental Status

Fudosteine is awaiting approval in Japan for the treatment of chronic respiratory disorders (14).

Manufacturer

SS Pharmaceutical Co., Ltd. (JP); licensed to Yoshitomi Pharmaceutical Industries, Ltd. (JP) for codevelopment and marketing.

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