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Testicular toxicity induced by a triple neurokinin receptor antagonist in male dogs

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ABSTRACT

Mechanism mediating the testicular toxicity induced by CS-003, a triple neurokinin receptor antagonist, was investigated in male dogs. Daily CS-003 administrations showed testicular toxicity, such as a decrease in the sperm number, motility and prostate weight; and an increase in sperm abnormality, accompanying histopathological changes in the testis, epididymis and prostate. A single CS-003 administration suppressed plasma testosterone and LH levels in intact and castrated males. The suppressed LH release was restored by GnRH agonist injection, suggesting that pituitary sensitivity to GnRH is not impaired by CS-003. Treatment with SB223412, a neurokinin 3 receptor antagonist, caused a similar effect to CS-003, such as toxicity in the testis, prostate and epididymis and decreased plasma level of LH and testosterone. In conclusion, CS-003-induced testicular toxicity is caused by the inhibition of neurokinin B/neurokinin 3 receptor signaling probably at the hypothalamic level in male dogs.

1. Introduction

Neurokinins (also known as tachykinins) are members of a family of small peptides with a common C-terminal sequence of Phe-X-Gly-Leu-Met-NH_2 [1]. Substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) represent the principal neurokinin peptides in mammalian tissues. Several molecular subspecies, including neuropeptide K (NPK) and neuropeptide gamma (NPG), N-terminally elongated forms of NKA, have also been reported [2,3]. The SP and NKA precursor proteins are encoded by the same gene, called preprotachykinin 1, while another gene, preprotachykinin 2, encodes NKB precursor [4]. Neurokinins are widely distributed in the central and peripheral nervous systems [3] with various biological functions, such as neurotransmission [3], vasodilation, smooth muscle contraction [5] and nociception [6]. They have also been reported to contribute to the regulation of hypothalamo-pituitary functions [7]. Biological actions of neurokinins are mediated by three types of G-protein coupled neurokinin receptors. SP interacts preferentially with NK1 receptor, NKA with NK2 receptor and NKB with NK3 receptor [8]. All types of NK receptors are distributed throughout the central and peripheral nervous system [1,9,10].

A drug, CS-003, used in the present study is an orally active triple neurokinin receptor antagonist, which was developed for the treatment of respiratory diseases, such as asthma and chronic obstructive pulmonary disease, since neurokinin receptors produce vascular hyperpermeability, bronchoconstriction, airway hyperresponsiveness, cough and mucus secretion [11]. This drug has been shown to have *in vitro*, as well as *in vivo* antagonistic activity against NK1, NK2 and NK3 receptors [12,13]. In guinea pigs, CS-003 inhibited SP-induced tracheal vascular permeability and NKA- or NKB-induced bronchoconstriction. In the course of the preclinical toxicity evaluation of the drug, testicular toxicity was found in the toxicity study with repeated administration in dogs. Another triple neurokinin receptor antagonist, SCH206272, has been reported to cause testicular toxicity in dogs [14] as found in the present study. The mechanism underlying the toxicity, however, remains unknown.

The present study aims to clarify the mechanism underlying CS-003 testicular toxicity in male dogs with hormone analysis. Furthermore, we examined the effect of NK3 receptor antagonist, SB223412 [15], to identify the receptor subtype involved in CS-003 testicular toxicity.

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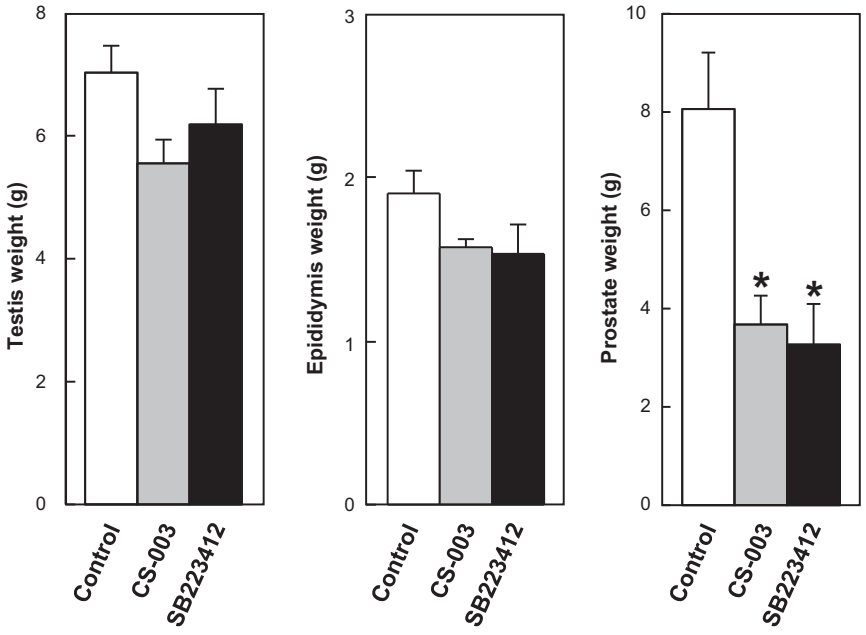

Fig. 1. Organ weight of testis, epididymis and prostate in CS-003 (triple neurokinin receptor antagonist,) or SB223412 (neurokinin 3 receptor antagonist)-treated intact male dogs. Tissues were collected from animals that received daily oral administration of CS-003 or SB223412 for 14 days. Values are mean ± S.E.M. ($n = 6$ for control, $n = 3$ for drug-treated groups). *$P < 0.05$, vs. control.

2. Materials and methods

2.1. Animals

Sexually matured male beagle dogs, aged 11–13 months (NARC Corporation, Chiba, Japan), were used. They were kept under a constant temperature (22 ± 2 °C), light/dark cycles (12 h light and 12 h darkness, lights on at 07:00) with about 300 g of food (Certified Canine Diet 5007, PMI Nutrition International, Richmond, USA) and free access to water. This study was conducted according to the in-house guidelines provided by the Institutional Animal Care and Use Committee of Daiichi Sankyo Co. Ltd.

2.2. Drugs

Triple neurokinin receptor antagonist, CS-003, used in the present study is [1-{2-[(2R)-(3,4-dichlorophenyl)-4-(3,4,5-trimethoxybenzoyl)morpholin-2-yl]ethyl}spiro[benzo[c]thiophene-1(3H),4′-piperidine]-(2S)-oxide hydrochloride]; and neurokinin 3 receptor antagonist, SB223412, is [(S)-(-)-N-(α-ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide]. Both of these were synthesized by Sankyo Co. Ltd. (Tokyo, Japan). A GnRH agonist, fertirelin acetate, was purchased from Fujita Pharmaceutical Co. Ltd. (Tokyo, Japan).

2.3. Drug administration

A gelatin capsule (1/4 oz; Torpac Inc., Fairfield, USA) filled with the drugs was given orally once a day at 09:00 h at doses of 100 mg/kg for CS-003 and 300 mg/kg for SB223412. The dose of SB223412 was determined by comparing the receptor binding affinity between CS-003 and SB223412 [13,16]. Animals in the control group were given empty capsules in the same manner as in the treated groups.

2.4. Effects of 14-day administration of CS-003 or SB223412 on reproductive organs and spermatogenesis

CS-003, SB223412 or an empty capsule was administered to intact male dogs for 14 days ($n = 3$). Animals were then anesthetized with sodium pentobarbital (Somnopentyl: Kyoritsu Seiyaku Corporation, Tokyo, Japan) for autopsies. Organ weight of the testis, epididymis and prostate were measured and histopathological examination was conducted. Sperm examinations including sperm motility, the number of sperm in the testis and sperm morphology were also performed.

2.5. Effects of CS-003 or SB223412 administration on plasma levels of reproductive hormones

CS-003 or an empty capsule was given once to intact and castrated male dogs ($n = 3$). Blood samples (2 ml) were collected from the jugular vein of the animals at 20 min intervals until 8 h after administration without anesthesia. SB223412 or an empty capsule was also given once to intact male dogs ($n = 3$). Blood samples were collected from the jugular vein at 1 h intervals until 8 h after treatment. Plasma testosterone (only in intact males), LH or FSH levels were determined by radioimmunoassay.

2.6. Effects of GnRH agonist on suppressed LH secretion after single CS-003 administration

Fertirelin acetate, a GnRH agonist, was injected intramuscularly at a dose of 50 μg/body 4 h after the single administration of CS-003 to determine if CS-003 changes pituitary sensitivity to GnRH ($n = 3$). Blood samples were collected at 20 min intervals until 8 h after CS-003 administration. Plasma LH or FSH levels were determined by radioimmunoassay.

2.7. Histopathological examination and sperm examinations

The testes were fixed in Bouin's solution and the epididymis and prostate were fixed in 10% neutral buffered formalin. Paraffin sections (4 μm) were made with a microtome and stained with hematoxylin and eosin. The number of sperm in the testis was determined by the sperm head count method [17]. Briefly, after removal of the tunica albuginea, the testis was placed in a tube with distilled water. They were homogenized for 30 s and sonicated for 3 min. The homogenate was placed on a hemacytometer to count the number of sperm heads under a microscope. The sperm was taken from the cauda epididymis and placed on a sperm counting chamber (Kitazato Supply Company, Limited, Shizuoka, Japan) to observe sperm motility. For sperm morphology, sperm sample was stained with Wright stain solution and observed under a microscope.

2.8. Hormone assays

Hormone assays were conducted at Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology, Tokyo, Japan. Plasma LH and FSH concentrations were determined by heterologous radioimmunoassay as described by Nakada et al. [18]. Dog LH (LER-1685-1) was used as a reference standard in the LH assay. The intra- and interassay coefficients of variation were 5.8 and 14.3%, respectively. Dog FSH (LER-1685-3A) was used as a reference standard in FSH assay. The intra- and interassay coefficients of variation were 3.1 and 10.8%, respectively. Plasma testosterone concentrations were assayed by radioimmunoassay as described by Taya et al. [19]. The intra- and interassay coefficients of variation were 3.9 and 5.0%, respectively.

2.9. Data analysis

Results are expressed as mean ± S.E.M. The results on the organ weight and sperm parameters (number of sperm in the testis, sperm motility and the sperm abnormality rate) in control animals of the CS-003 and SB223412 studies were combined, since the two control groups received similar treatments and no statistical difference was detected between the two groups. Significance of the difference