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ORIGINAL ARTICLE

Pharmacology and structures of the free base of the anaesthetic kazcaine and its complex with β-cyclodextrin

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Abstract The base form of the local anaesthetic kazcaine (BFK, [1-(2-ethoxyethyl)-4-ethynyl-4-benzoyloxypiperidine, $C_{18}H_{23}NO_3$]) and β-cyclodextrin (β-CD) co-crystallized as BFK:β-CD inclusion complex in 1:2 M ratio from a mixture of water and ethanol while the filtered mother liquor yielded crystals of free BFK. X-ray diffraction showed that the crystals of BFK and its inclusion complex with β-CD belong to monoclinic ($P2_1/c$) and triclinic ($P1$) space groups, respectively. The crystals of free BFK are stabilized by pairs of C–H···O, C–H···π and ≡C–H···O type interactions and van der Waals contacts. In the 1:2 BFK:β-CD complex the two β-CD molecules are in hydrogen-bonding contact with their primary hydroxyl groups, the 1-(2-ethoxyethyl)-4-ethynyl-piperidine moiety being located in one and the benzoyloxy group of BFK in the other β-CD. This crystal structure is of the channel-type, the β-CD molecules of the 1:2 BFK:β-CD complex interacting with their secondary hydroxyl groups. The pharmacological activities of the 1:2 BFK/β-CD inclusion complex have been determined in mice, rats, porpoises and rabbits and compare favourably with those of kazcaine, procaine, dicaine, lidocaine and trimecaine. The methods used include terminal (superficial), infiltration, conduction anaesthesia, and acute toxicity.

Keywords Kazcaine base · Cyclodextrin · Crystal structure · X-ray diffraction · Pharmacological properties · Anaesthetic

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Introduction

The cyclodextrins (α-CD, β-CD, γ-CD) are cyclic oligosaccharides containing six, seven or eight D-glucoses bonded with each other by α-1,4-glucosidic bonds [1, 2]. The external side of these macrocyclic molecules is hydrophilic and their inner cavity is hydrophobic [3, 4].

Cyclodextrin complexes with various physiologically active substances are of great pharmaceutical interest (see reviews [5, 6]). Inclusion complexes of drugs with CDs allow overcoming a number of problems of drugs concerning unpleasant taste and odour, instability, low solubility in water, and may improve some pharmacological properties by reducing toxicity [7, 8].

The local anaesthetic kazcaine [1-(2-ethoxyethyl)-4-ethynyl-4-benzoyloxy-piperidine hydrochloride] proved to be the most efficient among different synthesized 4,4-disubstituted derivatives of piperidine, which possess as substituent $C_2H_4OCH_3$, $C_2H_4OC_2H_5$, $C_3H_6OC_4H_9$ at atom N_1 and C≡CH, C≡CCH=CH_2, C≡CPh, COOH and OC-OCH_3, $OCOC_2H_5$, OCOPh at atom C_4 of the piperidine cycle [9, 10]. Kazcaine (Fig. 1) has been recommended for infiltration and conduction anaesthesia [11].

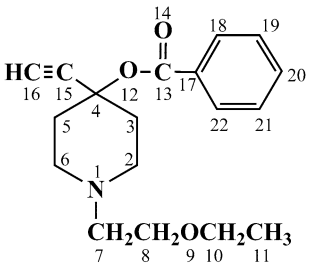

Fig. 1 Chemical structure of kazkaine-base (BFK) with atomic numbering scheme used in the text. Kazkaine is the hydrochloride of BFK with protonated N1 and additional chloride

Since kazcaine (the hydrochloride form) showed some toxicity and the base form of kazcaine (BFK) is only sparingly soluble in water, the synthesis of a new form of kazcaine-base with minimal side effects is desirable. For this purpose, the inclusion complex of BFK (Fig. 1) was co-crystallized with β-cyclodextrin (β-CD). Here we describe the crystal structures of BFK, its inclusion complex with β-CD, and pharmacological properties of the obtained 1:2 BFK:β-CD complex.

Materials and methods

Crystallization and X-ray crystallography

β-cyclodextrin from SIGMA has been used to produce the inclusion complex with BFK (synthesized as described in [9, 11]) by co-precipitation from aqueous-ethanol solution.

50 mL of a 20 mM aqueous solution of β-CD was added to 25 mL of a 40 mM ethanol solution of BFK, heated to 65 °C during 15 min and allowed to cool to room temperature. The formed precipitate was separated from the mother liquor, washed with distilled water and left at room temperature for 24 h to dry, yielding 1.437 g (86.7%) of white crystalline powder of β-CD inclusion complex with BFK. Of this complex, 0.6 g were dissolved in 30 mL aqueous-ethanol solution (5:1 v/v) at 65 °C, stirred for 15–20 min and then allowed to cool to room temperature. The β-CD inclusion complex with BFK crystallized after 2 days.

The above crystals were filtered from the solution, and pure BFK crystallized from this mother liquor after 3 days.

X-Ray measurements, determination and refinement of the crystal structures

Crystals of BFK and of the inclusion complex with β-CD were mounted on glass fibers with Kel-F90 grease. X-ray data collection was performed using a SMART CCD diffractometer (Bruker) with MoK_α radiation operating at 50 kV, 30 mA. For BFK and for the complex with β-CD 3319 and 16,612 unique reflections were measured in the θ range 1.15–26.37°, respectively. Data reduction was performed with the program SAINT [12], including semiempirical absorption correction with SADABS [13]. The crystals belong to monoclinic and to triclinic space groups $P2_1/c$ and $P1$, respectively (for details, see Table 1).

The crystal structures were determined by direct methods followed by Fourier syntheses and refined by full-matrix least-squares based on F^2 with the program SHELX [14], based on 291 parameters for BFK and 1,806 for the complex with β-CD, with no restraints for BFK and nine restraints for the complex with β-CD. The refinement using the observed ($I > 4\sigma(I)$) reflections converged at $R1 = 0.0382$ for BFK and at $R1 = 0.0778$ for the complex with β-CD. For the latter, water and guest molecules were located in difference Fourier maps and showed that a BFK:β-CD complex with stoichiometry 1:2 had crystallized. In the final structural model the oxygen atoms of water molecules are distributed over 22 positions of which 15 are fully occupied (1.00) and the occupancies of 7 are in the range of 0.22–0.50. Potential water oxygen atoms with occupancy less than about 0.20 were not considered meaningful. All non-hydrogen atoms were refined anisotropically. Except for those attached to the water molecules, all hydrogen atoms were added in the ideal positions (C–H, O–H distances at 0.979 and 0.820 Å, respectively) and refined as riding models.

Pharmacology

The current pharmacological studies were done in compliance with "The methodical recommendations for experimental studies on local anaesthetic medications" approved by the Pharmacological Committee of the Republic of Kazakhstan in 2000 and with "The Principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985). The animals from the Kazakh National Medical University (KNMU) vivarium that were used in the here described experiments are: white mice, white rats, porpoises and rabbits. Pharmacological tests have been carried out for the 1:2 BFK:β-CD complex at KNMU (Almaty, Kazakhstan). The methods used were terminal (superficial) anaesthesia, infiltration anaesthesia, conduction anaesthesia, and acute toxicity.

Results

Crystal structures

The base form of kazcaine

Many attempts to crystallize BFK failed but when the mother liquor of the crystallized 1:2 BFK:β-CD complex was filtered and stored at room temperature for some days,

Table 1 Crystal data and structure refinement of BFK and inclusion complex of the BFK with β-CD

Name	BFK	Complex BFK with β-CD
Chemical formula	$C_{18}H_{23}NO_3$	$2C_{42}H_{70}O_{35} + C_{18}H_{23}NO_3 + 17.5H_2O$
Formula weight	301.4	2886.61
Temperature (K)	133(2)	133(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/c$	$P1$
a (Å)	9.468(3)	15.299(4)
b (Å)	16.146(4)	15.470(4)
c (Å)	10.675(3)	15.590(4)
α (°)	90.000	104.548(6)
β (°)	90.061(7)	101.025(7)
γ (°)	90.000	104.067(7)
Volume (Å)^3	1632.0(7)	3337.1(2)
Z	4	1
D_c (g/cm^3)	1.227	1.436
Absorption coefficient (mm^{-1})	0.08	0.13
F (000)	648	1,506
Crystal size (mm)	0.62 × 0.41 × 0.02	0.46 × 0.31 × 0.30
θ range for data collection (°)	2.15–26.37	2.72–24.77
Index range	$-11 \le h \le 11$, $-20 \le k \le 20$, $-13 \le l \le 11$	$-17 \le h \le 17$, $-18 \le k \le 18$, $-18 \le l \le 18$
Reflections collected/unique	17,186/3,319	35,107/16,612
R_{int}	0.1294	0.0837
Data/restraints/parameters	3,319/0/291	16,612/9/1,806
Goodness-of-fit on F^2	0.995	0.993
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Final R indices [$I > 4\sigma(I)$]	$R1 = 0.0382$, $wR2 = 0.1019$	$R1 = 0.0778$, $wR2 = 0.2115$
R indices (all data)	$R1 = 0.0633$	$R1 = 0.1175$

free BFK crystallized in the monoclinic space group $P2_1/c$ (see "Crystallization and X-ray crystallography" section). Geometric parameters of the BFK molecule correspond to conventional values, and the bond lengths conform well to standard values [15]. The ethoxyethyl and benzoyloxy groups are in equatorial and the ethynyl group in axial positions. The piperidine cycle assumes the conformation of a slightly distorted $^NC_4$ chair with Cremer–Pople parameters $Q = 0.589$ Å, $\theta = 2.70°$, $\varphi = 343.15°$. The N(l) and C(4) atoms are 0.692(2) and −0.673(2) Å, respectively, from the other four atoms that are coplanar within 0.005 Å.

There are two intramolecular C–H···O hydrogen bonds in the molecule. The ethoxyethyl group adopts an extended conformation that is stabilized by C(2)–H_A···O(9), and the orientation of the benzoyloxy group is stabilized by C(3)–H_B···O(14) (Fig. 2).

In the crystals (Fig. 3) two BFK form a centrosymmetric dimer and interact through pairs of short intermolecular –C≡C(16)–H···O(9) hydrogen bonds with H···O and C···O distances of 2.30 and 3.23 Å, respectively. Symmetry related dimers are at van der Waals contacts in the crystal and interact through hydrogen bonds C(5)–H_A···O(14) and C(10)–H···π(C(17)) with long H···O distances of 2.72 and 2.86 Å, respectively.

In the 1:2 BFK:β-CD inclusion complex the BFK has a different conformation compared to the free form (Fig. 2). The ethoxyethyl group adopts a bent conformation stabilized by a C(10)–H_B···N(1) hydrogen bond, and the benzoyl group is flipped by ~90° about the C(4)–O(12) bond and stabilized by a C(5)–H_B···O(14) hydrogen bond (Fig. 2, right). The piperidine ring is in a similar chair conformation as in the free form with Cremer–Pople parameters $Q = 0.574$ Å, $\theta = 1.59°$, $\varphi = 5.38°$. The N(l) and C(4) atoms are at 0.683(18) and −0.683(19) Å, respectively, from the least-squares plane of the other four atoms that are within 0.005 Å.

Crystal structure of the 1:2 BFK:β-CD complex

The asymmetric unit of the 1:2 BFK:β-CD complex in space group $P1$ comprises two β-CDs, one BFK and 17.5 water

molecules. The β-CD molecules are stacked along the crystallographic *c*-axis in the alternating head-to-head and tail-to-tail channel mode (Fig. 4) as frequently observed in β-CD crystal structures [16]. The BFK molecules are located in the β-CD channels in head-to-tail mode, and the presence of hydrophobic aryl and ethoxyethyl groups at both ends does not allow any water molecules to enter the channels. Partly disordered water molecules fill 22 sites located in the space between the β-CD channels, thereby contributing significantly to the stability of the crystal structure by means of a large number of hydrogen bonds with hydroxyl groups of the β-CD molecules.

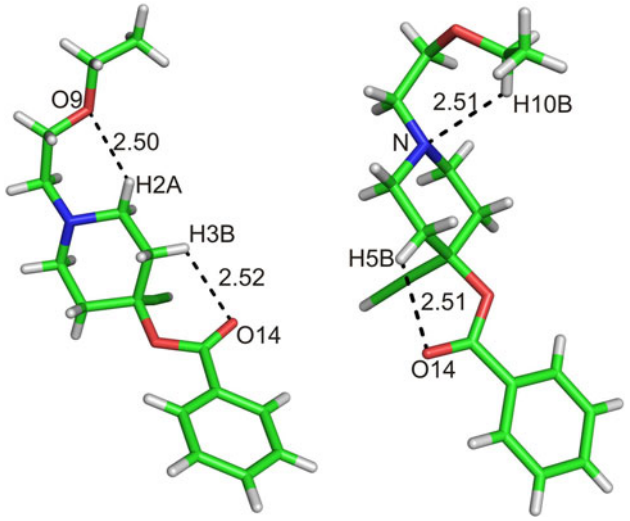

Fig. 2 Intramolecular hydrogen bonds (*black dashed lines*) in free BFK (*left*) and in complex with β-CD (*right*). The views are roughly similar with respect to the piperidine ring. Note differences in the orientations of the benzoyl groups and in the folding of the ethoxyethyl groups (H···O distances are in Å)

The two crystallographically independent β-CD molecules A and B form a head-to-head dimer with 28 direct O–H···O hydrogen bonds connecting their secondary faces (mean O···O distance 2.97 Å within the range 2.70–3.25 Å). On the other side, tail-to-tail dimers are formed by only three direct O–H···O hydrogen bonds connecting their primary faces formed by O6 hydroxyl groups (within the range 2.68–2.81 Å) and three additional water-mediated hydrogen bonds O–H···O_W–H···O (in the range 2.73–3.20 Å) as frequently observed for this kind of supramolecular complexes [17, 18].

Geometry of the β-CD molecules in the complex

Each glucose residue of β-CD adopts the usual $^4C_1$ chair conformation, and the overall β-CD molecule has an approximate 7-fold axis. The geometric parameters for the two independent β-CD molecules are listed in Table 2. The

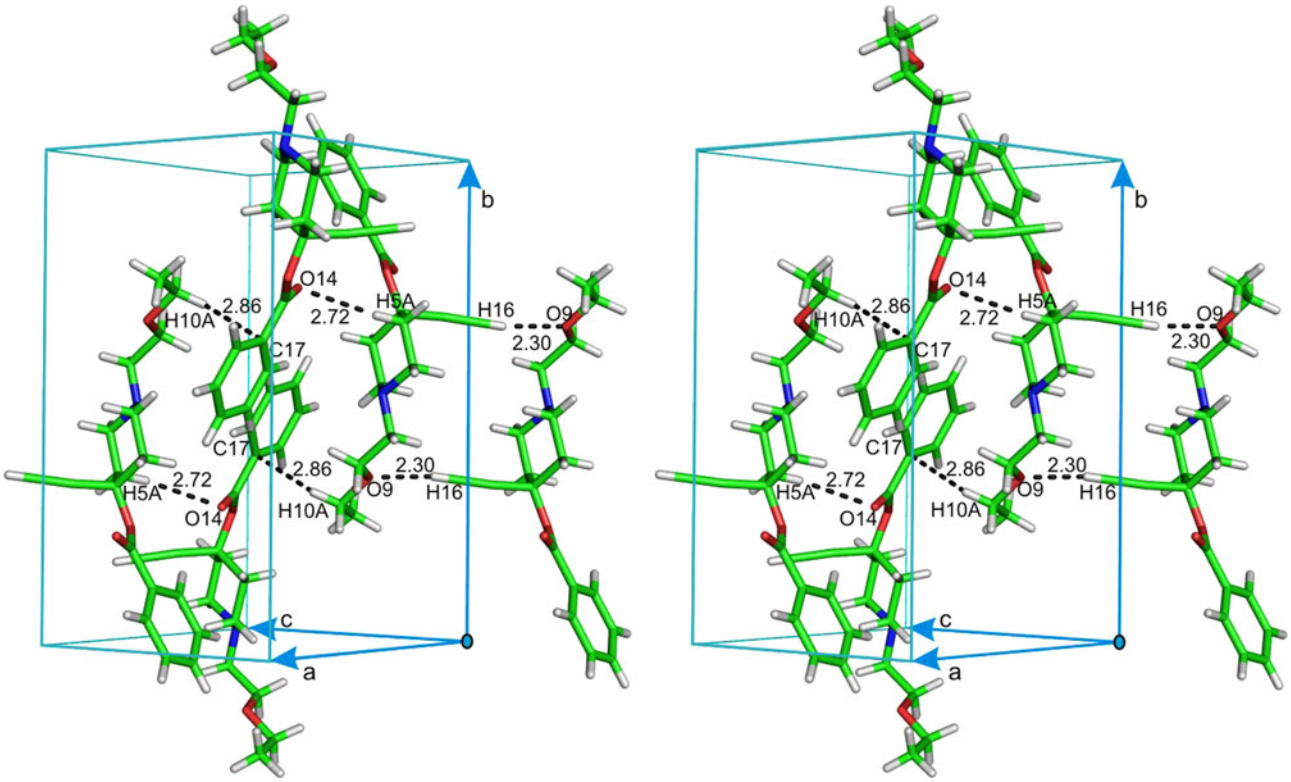

Fig. 3 Stereo view of the crystal structure of kazcaine-base with intermolecular C–H···O and C–H···π hydrogen bonds indicated by *black dashed lines* (distances H···O, H···C in Å)

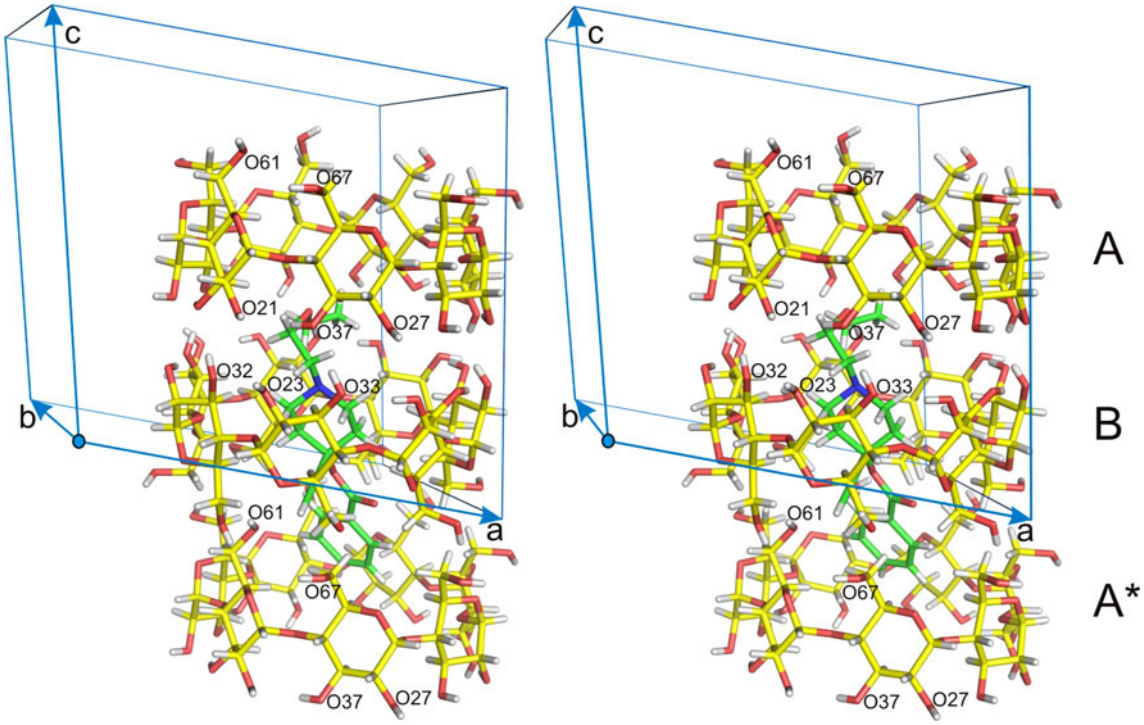

Fig. 4 Stereo view of the inclusion complex kazcaine-base · 2 *β*-CD·17.5 H_2O. *β*-CD in *yellow*, O *red*, H *white*, and kazcaine *green*, O *red*, N *blue*, H *white*, the water molecules are omitted for sake of clarity (Color figure online)

O(4*n*) atoms (*n* denoting the number of the glucose in the macrocycle) of molecules A and B are almost co-planar, with the mean deviation from the least-squares plane being 0.05 and 0.04 Å for molecules A and B, respectively, the angle between the two planes being only 1.2°.

The orientation of the C(6*n*)–O(6*n*) bond is described by torsion angles C(4*n*)–C(5*n*)–C(6*n*)–O(6*n*) and O(5*n*)–C(5*n*)–C(6*n*)–O(6*n*), Table 2. Most of the primary hydroxyl groups have the (+)gauche, (−)gauche orientation and point "away" from the cavity as indicated by the corresponding angles in the ranges 50.22°–62.17° and −59.25° to 80.42°, respectively.

Geometry of inclusion

The 1:2 BFK:*β*-CD complex stoichiometry is less common and formed when the guest molecule can not be totally included into an individual *β*-CD cavity. The main part of BFK is located in *β*-CD molecule B and comprises the piperidine moiety, the carboxyl, ethynyl and the ethoxyethyl groups, the latter also being partly included in *β*-CD molecule A that is hydrogen bonded to molecule B by secondary hydroxyl groups. The phenyl group of BFK is harbored mainly by *β*-CD molecule A* that is hydrogen bonded to molecule B through primary O(6) hydroxyl groups, see Fig. 4. The interactions between *β*-CD and BFK are mainly of van der Waals type involving hydrogen atoms, and C–H···O hydrogen bonds are formed between BFK O(14) with H···O distances within the range 2.68–2.97 Å and C(64)–H, C(55)–H of *β*-CD molecule B and C(67)–H of molecule A*. The ethynyl group is not hydrogen bonding to any oxygen atom, except to O(67) from *β*-CD molecule A*, with H···O distance of 2.69 Å.

Pharmacological studies

Terminal (superficial) anaesthesia

The ability of compounds to fulfil superficial anaesthesia was studied on a rabbits' eye cornea by Rene's method [19], which is based on the summation principle of threshold mechanical irritations rhythmically applied to a cornea. During the experiment the strength of anaesthesia (the maximum index is 1,300), the duration of the total anaesthetic effect and the general duration of the effect were observed. The activity of the examined compound was compared to the standard medication dicaine (tetracaine) and kazcaine (Table 3).

Infiltration anaesthesia

To measure the local anaesthetic activity at infiltration anaesthesia the intradermal method of Bulbring–Wade [19] was used. Experiments were carried out on porpoise males

Table 2 β-CD macrocycle characteristics

Residue	D^a (Å)	Φ^b(°)	d^c (Å)	D^d (Å)	Torsion angle (°)	
					C4–C5–C6–O6	O5–C5–C6–O6
Molecule B						
G1	4.40	130.69	−0.08	2.79	59.45	−62.83
G2	4.33	126.43	0.08	2.82	−172.26	69.09
G3	4.40	127.69	−0.01	2.85	54.41	−65.51
G4	4.36	130.54	−0.05	2.73	55.61	−66.75
G5	4.37	128.33	0.02	2.75	62.08	−59.48
G6	4.31	127.24	0.02	2.76	52.14	−61.29
					56.99	−80.42
G7	4.40	128.84	0.01	2.73	53.57	−70.74
Molecule A						
G1	4.33	125.83	−0.08	2.76	−172.07	70.39
G2	4.48	133.37	0.02	2.69	60.09	−61.21
G3	4.29	126.97	0.10	2.85	−176.36	63.36
					50.22	−70.06
G4	4.41	125.07	−0.08	2.90	62.17	−59.25
G5	4.37	132.17	−0.03	2.75	57.51	−63.59
G6	4.40	128.5	0.06	2.80	50.71	−71.73
G7	4.37	125.83	−0.002	2.78	57.94	−61.95

[a] Distance between atoms $O4n\cdots O4(n+1)$

[b] Angles between atoms $O4(n-1)\cdots O4n\cdots O4(n+1)$

[c] Deviations (Å) from the least-squares optimum plane of the seven O4*n* atoms

[d] Intramolecular hydrogen-bond distance between $O3n\cdots O2(n+1)$

Table 3 Anaesthetic activity of the 1:2 BFK:β-CD complex and reference preparations at terminal anaesthesia. (M ± m)

Preparations	Rene index	Complete anaesthesia (min)	Total duration of effect (min)	Rene Index	Complete anaesthesia (min)	Total duration of effect (min)
Concentration (%)	1	1	1	5	5	5
BFK:β-CD[c]	441.6 ± 16.4[a]	0	42.8 ± 1.8[b]	636.2 ± 8.6[b]	31.0 ± 2.6[b]	68.5 ± 2.53[b]
Kazcaine	217.2 ± 18.75	0	22.0 ± 1.3	575.0 ± 30.46	14.5 ± 1.8	35.7 ± 1.75
Dicaine (tetracaine)	1300 ± 0	Longer than 50	60 and longer	_	_	_

[a] Deviations in relation to tetracaine and

[b] Kazcaine are statistically authentic at $p < 0.001$

[c] By mass BFK is 1/10 of the complex

Table 4 Anaesthetic activity of the 1:2 BFK:β-CD complex and reference preparations at infiltrational anaesthesia and at conductive anaesthesia, (M ± m)

Preparations	Concentration (%)	By the Bulbring–Wade method			"Draw a tail aside"mode		
		BFK:β-CD[c]	Kazcaine	Procaine	BFK:β-CD[c]	Kazcaine	Procaine
Complete anaesthesia (min)	0.5	63.3 ± 2.9	26.3 ± 2.9	13.3 ± 1.1	106.1 ± 2.0[a]	74.4 ± 11.1	24.2 ± 3.9
Total duration of effect (min)	0.5	108.4 ± 2.7	82.9 ± 3.6	30.0 ± 1.3	118.9 ± 6.8[a]	97.6 ± 6.3	33.3 ± 11.2
Complete anaesthesia (min)	1	121.3 ± 4.3[a]	67.4 ± 1.9	20.1 ± 1.6	137.1 ± 3.9[b]	103.4 ± 11.1	34.2 ± 6.9
Total duration of effect (min)	1	136.1 ± 1.7[a]	101.9 ± 3.5	42.0 ± 1.2	147.5 ± 6.7[a]	119.6 ± 5.5	41.3 ± 4.6

[a] Deviations in relation to reference preparations are statistically authentic at $p < 0.001$

[b] In relation to kazcaine are authentic at $p < 0.01$

[c] By mass BFK is 1/10 of the complex

with weight of 200–250 g. The depth of anaesthesia (the maximum index is 36), duration of full anaesthesia and general duration of the anaesthetical effect were determined. The activity of the compound was compared to standard medications with novocaine (procaine) and kazcaine (Table 4).

Table 5 Acute toxicity of observable and reference preparations tested on white mice at hypodermic injections

Preparation	LD50 (mg/kg)	p
Complex of the 1:2 BFK:β-CD[a]	590.0 ± 11.3	
Procaine	486.0 ± 8.8	p_1
Lidocaine	249.5 ± 18.4	p_2
Trimecaine	378.2 ± 17.4	p_3
Kazcaine	529.3 ± 7.1	p_4

Note: Deviations for complex of 1:2 BFK:β-CD in relation to reference preparations are statistically authentic at $p1$, $p2$, $p3$, $p4 < 0.001$

[a] By mass BFK is 1/10 of the complex

Conduction anaesthesia

A modified "draw a tail aside" method [20] was used on outbred rats (males with weight of 200–250 g). Solutions of each concentration given in Table 4 were tested on six animals. At first, the pain threshold was determined. Later, tailheads of the examined rats were evenly pricked from four sides with observable and reference preparations (1 mL each). Animals from the set were treated in the same way, but with normal saline solution. The irritation was applied 1 cm distal from the injection. After injection of observable and reference preparations, repeated tests were carried out with a certain time slice. Doubling of the latent period of a "draw a tail aside" reflex was counted as total anaesthesia (Table 4).

Acute toxicity

Acute toxicity of substances was determined by single abdominal and hypodermic injections to white outbred mice (6–8 species in screening with weight of 17–23 g). The treated animals were supervised continuously within several hours and further 6 days after injections and compared with the untreated animals. Behavior changes, reflectory breath excitability, rate of development and regress of external poisoning indications and mortality were registered. Data were processed accordingly to Litchfield, Wilkinson and Berensz [21], and half-lethal doses (LD50) were calculated (Table 5).

Discussion

Crystal structures

Looking at Fig. 4, it appears that BFK were better included by β-CD if it would move "up" to fit deeper into the cavity formed by molecules A and B. This is obviously avoided for steric reasons because the ethynyl group sticks out towards the primary side of molecule B, and the phenyl group of the benzoyl group pushes β-CD molecule A* to the "right" which is possibly easier (because there are fewer O6–H···O6 hydrogen bonds) than to push one β-CD relative to the other if they are connected by many more hydrogen bonds formed by the secondary hydroxyl groups, as between β-CD molecules A and B.

In solution, a comparable inclusion complex could be formed by BFK and two β-CD molecules stabilized by hydrogen bonds between the primary O6–H groups forming the host for BFK because only then the hydrogen-bonded β-CD can shift laterally to accommodate the sterically demanding BFK. We anticipate that this complex will look similar to the β-CD molecules B and A* with BFK included, its ethoxyethyl moiety partially protruding from the cavity formed by the β-CD molecules.

However, it could also be that in solution a 1:1 complex is formed by β-CD molecule B and BFK as shown in Fig 4, the benzoyl group being exposed to solvent and not included by a second β-CD.

Pharmacological studies

1 and 5% aqueous complexes of 1:2 BFK/β-CD were tested for terminal anaesthesia, Table 3. The trials showed that the complex of 1:2 BFK/β-CD in both tested concentrations has defined local anaesthetic activity. As seen in Table 3, 1% of the complex of 1:2 BFK:β-CD in water caused 2-fold anaesthesia effect and about twice prolonged duration of the general anaesthesia compared to the anaesthetic kazcaine. Compared to BFK, the 1:2 BFK:β-CD complex dissolved at 5% concentration in water also showed improved local anaesthetic effect (1.1 times Rene's index), prolongation of total anaesthesia (2.2 times) and notable general prolongation effect (1.9 times). It must be noticed that the tested complexes of 1:2 BFK:β-CD did not show any irritation effect, while kazcaine caused insignificant irritation when applied to rabbit's eye tissue, gave rise to hyperemia of conjunctiva and of eyelids' edges of nictitating membranes within 20 min as well as in closing of eyelids within 5–7 min after application, ("+"—on the scale of Setnikara) [19].

The same complexes were tested for infiltrational anaesthesia with 0.5 and 1% solutions (Table 4). The complex of 1:2 BFK:β-CD dissolved at 0.5% concentration in water showed 4.7-fold prolongation of complete anaesthesia compared to procaine (2.4-fold compared to kazcaine) and in 3.6 times longer activity than procaine (at $p < 0.001$). With 1.0% 1:2 BFK/β-CD complex in water the duration of complete anaesthesia was as high as 121 min, which excels given parameter for procaine six times and that of kazcaine 1.7 times. The total duration of the effect was 3.2 times and

1.3 times longer compared to procaine and kazcaine, respectively.

During conductive anaesthesia experiments (Table 4) the 1:2 BFK:β-CD complexes also showed higher activity than reference medications. At 0.5% concentration in water the 1:2 BFK:β-CD complex provided complete anaesthesia in all screens, with 4.4- or 1.4-fold prolongation compared to procaine or kazcaine, respectively. The total duration of the anaesthetic effect was also extended considerably compared to procaine (3.5 times) and insignificantly compared to kazcaine (1.2 times). Increase of the 1:2 BFK:β-CD complex to 1.0% concentration showed profound local anaesthetic activity. The duration of complete anaesthesia was 4 or 1.3 times longer compared to procaine and kazcaine, respectively. The total duration of the anaesthetic effect was 1.2 times longer than for kazcaine.

According to acute toxicity, toxic reactions were of the same character, the higher the dose, the faster poisoning became evident. Intoxication declared itself 20–30 min after injection. The initial stage started with general oppression, yielding in deferred response, absence of reflex to exogenous irritants, dyspnea that later developed into a short period of motional excitation followed by muscular twitching and clonico-tonic spasms. Mice accepted a lateral position, breath became rare, arrhythmic. The death came from primary respiratory standstill in 30–90 min after injection. The survived mice recovered from stagnation in 2–2.5 h and were as active as untreated mice by the end of first day.

Data on half-lethal doses (LD50) are presented in Table 5. The complex of 1:2 BFK:β-CD appeared to be less toxic than reference preparations and its effect is approximately comparable with that of kazcaine.

Conclusions

The complex 1:2 BFK:β-CD has noteworthy anaesthetic effects at infiltrational and conductive anaesthesia, which is higher compared to reference preparations, and it is also of great importance that this complex is less toxic. It needs to be mentioned that the solutions used for the pharmacological studies contained BFK with an amount corresponding to only one 10th of the complex 1:2 BFK:β-CD (see footnotes Tables 3, 4, 5). In this connection 1:2 BFK:β-CD can be recommended for extensive clinical research with a wider range of doses.

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