Determination of the Nateglinide Polymorphism Structure and Reduction in the Blood Glucose Level

Li Gang[1], Chen Jiaying[2], LÜ Guanglie[3], Xu Qunwei[2]

(1. Testing & Analysis Center, Nanjing Normal University, 210097, Nanjing, China)
(2. Jiangsu Institute of Materia Medica, 210009, Nanjing, China)
(3. Testing & Analysis Center, Zhejiang University, 310028, Hangzhou, China)

Abstract: The nateglinide polymorphism structure and the effect on reducing the level of blood glucose are determined. The results indicate that nateglinide has three crystal forms, B-form, H-form and S-form. H-form belongs to the triclinic crystal system with the lattice constant $a = 1.769\,9(7)$ nm, $b = 2.719\,1(8)$ nm, $c = 1.267\,0(6)$ nm, $\alpha = 61.75(3)°$, $\beta = 73.11(6)°$, $\gamma = 66.77(7)°$, $V = 4.895$ nm^3, $Z = 10$. S-form belongs to the orthorhombic crystal system with the lattice constant $a = 2.317\,8(6)$ nm, $b = 2.533\,2(5)$ nm, $c = 0.653\,1(2)$ nm, $\alpha = \beta = \gamma = 90°$, $V = 3.835$ nm^3, $Z = 8$. S-form Nateglinide can significantly reduce the level of blood glucose of administration in high glucohemia mice like the H-form. Especially after 40 min the efficacy is much better.

Key words: nateglinide, polymorphism, X-ray powder diffraction, lattice parameters, blood glucose level
CLC number R917, **Document code** A, **Article ID** 1001-4616(2005)03-0065-04

那格列奈的多晶结构与降血糖作用

李钢[1], 陈家英[2], 吕光烈[3], 徐群为[2]

(1. 南京师范大学分析测试中心,210097, 江苏,南京)
(2. 江苏省药物研究所,210009, 江苏,南京)
(3. 浙江大学分析测试中心,310028, 浙江,杭州)

[**摘要**] 用 X 射线衍射法对那格列奈的多晶型结构进行了测定,并进行了动物模拟降血糖试验. 结果表明:那格列奈具有 B 晶型、H 晶型 和 S 晶型三种结构. H 晶型为三斜晶系,晶胞参数 $a = 1.769\,9(7)$ nm, $b = 2.719\,1(8)$ nm, $c = 1.267\,0(6)$ nm, $\alpha = 61.75(3)°$, $\beta = 73.11(6)°$, $\gamma = 66.77(7)°$, $V = 4.895$ nm^3, $Z = 10$. S 晶型为正交晶系,晶胞参数 $a = 2.317\,8(6)$ nm, $b = 2.533\,2(5)$ nm, $c = 0.653\,1(2)$ nm, $\alpha = \beta = \gamma = 90°$, $V = 3.835$ nm^3, $Z = 8$. 小白鼠模拟降糖试验表明:与目前上市的 H 晶型相同,S 晶型也具有很好的降血糖作用,特别是在用药 40 min 时,效果更显著.

[**关键词**] 那格列奈,多晶型,X 射线衍射,晶格参数,血糖值

0 Introduction

Type II diabetes is the most common form of diabetes and it occurs in people of all ages and races. In type II diabetes, either the body does not produce enough insulin or the cells ignore the insulin. Insulin is necessary for the body to be able to use glucose. Glucose is the basic fuel for the cells in the body, and insulin takes the glucose from the blood into the cells. When glucose builds up in the blood instead of going into cells, it can

Received date: 2005-03-01.
Foundation item: Supported by the Natural Science Foundation of Jiangsu Province (BK2001111) and the Key Lab of Nanjing Normal University (1812200024).
Biography: Li Gang, born in 1959, Associate professor, Majored in X-ray powder diffraction analysis. E-mail: ligang1@njnu.edu.cn

cause two problems: Right away, cells may be starved for energy. Over time, high blood glucose levels may hurt ones eyes, kidneys, nerves or heart. Nateglinide is a novel oral hypoglycemic agent for the management of type II diabetes. The drug has a rapid onset of action, short half-life, and short duration of action[1]. When administered prior to a meal, nateglinide restores phase I insulin secretion, which is lost in type II diabetics.

In the last articles, we first reported a new crystal form of nateglinide named S-Form[2] and the stability of three forms of nateglinide was also determined[3].

In this article, crystals structures of H-form and S-form nateglinide were determined, and their medicine efficiency was also tested and compared.

1 Materials and Methods

Three crystal forms of nateglinide were obtained from Jiangsu Institute of Materia Medica, China. S-form could be obtained from recrystallization of crude nateglinide in a special solvent. Crystals, which were in B-or H-form, could be changed to S-form in methanol. When heated H-form to melt, and cooled to r. t. slowly, S-form could be obtained, too.

The X-ray powder diffraction patterns of three crystal forms of nateglinide were obtained with a Rigaku Corporation D/max-rC rotating anode X-ray powder diffractometer using a copper target, equipped with a scintillation counter, a graphite crystal monochrometer. The aperture of the divergence, scattering and receiving slits were 1°, 1°and 0.30 mm respectively. The scan speed was 3°/min, over the range from 3°to 40°(2θ), in step scan mode increasing at a step size of 0.02°, operating at 40 kV and 100 mA. Powder samples were contained in a glass dish after being smoothed with a glass slide. The D-spacing data of structure analysis were obtained with a Rigaku D/max-2550/PC rotating anode X-ray powder diffractometer with Cu K alpha1 radiation, equipped with the parallel optical path system of multi-layer focus mirror, operating at 40 kV and 300 mA. And the Jade application software program was used to determine the crystal systems and lattice parameters of H-form and S-form, respectively.

80 ICR mice (about 22 - 25 g, male: female = 1: 1, Manufacture Licence Number: SCXK (Jiangsu) 2002 - 0031), which was divided to four groups, used to test and compare the medicine efficiency of H-form and S-form nateglinide. No. 1 is normal group, No. 2 is model group, No. 3 and No. 4 were fed H-form and S-form nateglinide, respectively. The glucose test kit is from Shanghai Rongsheng Co. (No. 20020102). The high glucohemia mice by injecting (ip.) the 2 g · kg^{-1} glucose were administrated of S-form Nateglinide 100mg · kg^{-1}. Then the values of blood glucose were observed after 20, 40 and 60 min of administration, respectively. Using the same method, the efficiency of H-form were tested.

2 Results and Discussion

X-ray powder diffraction analysis indicated three kinds of crystal structures existed in nateglinide and their patterns are shown in Fig. 1, 2, 3. In the XRD patterns, there are two strongest lines at 2θ values of 4.78° and 13.94° for the B-form, but no reflections for H-or S-form at the positions. As for the case of the H-form crystal, the lines at 19.54° and 19.74° are characteristic. On the other hand, in the XRD pattern of the S-form crystal, there is only one strongest line at 3.78°, showing a very strong orientation in the crystal particles. The main XRD date of three crystal forms are given in Table 1, 2, 3.

Table 1 The strongest lines of B-form nateglinide

2θ/°(meas.)	3.76	4.78	5.06	6.04	13.94	16.46	17.8	18.92	20.14
d values/nm	2.35	1.85	1.74	1.46	0.63	0.54	0.50	0.47	0.44
I/I_0	44	100	65	28	91	32	30	31	35

Table 2 The strongest lines of H-form nateglinide

2θ/°(meas.)	5.44	8.10	11.48	13.12	15.18	15.94	16.20	19.54	19.74
d values/nm	1.62	1.09	0.77	0.67	0.58	0.56	0.55	0.45	0.45
I/I_0	56	28	32	54	60	81	55	98	100

Table 3 The strongest lines of S-form nateglinide

2θ/°(meas.)	3.78	7.56	8.30	11.06	15.58	16.98	18.68	19.94	20.64
d values/nm	2.33	1.17	1.06	0.80	0.568	0.522	0.475	0.445	0.430
I/I_0	100	12	3	3	5	3	3	3	3

According to their XRD date, chemical composition ($C_{19}H_{27}NO_3$) and densities (1.04 for H-form, 1.09 for S-form), the lattice parameters of H-and S-form were determined. It indicated that H-form belongs to the triclinic crystal system with the lattice constant a = 1.769 9(7) nm, b = 2.719 1(8) nm, c = 1.267 0(6) nm, α = 61.75(3)°, β = 73.11(6)°, γ = 66.77(7)°, V = 4.895 nm^3, Z = 10. S-form belongs to the orthorhombic crystal system with the lattice constant a = 2.317 8(6) nm, b = 2.533 2(5) nm, c = 0.653 1(2) nm, $\alpha = \beta = \gamma$ = 90°, V = 3.835 nm^3, Z = 8.

The effect on reducing the level of blood glucose is related with nateglinide crystal structure. The known B-form crystal suffers from the problem of instability, especially in the process of mechanical grinding[4]. Polymorphs are regarded as thermodynamically different phases, and they possess different melting points, different heats of fusion, and different dissolution rates. Differences in properties can affect bioavailability and effective clinical use. So it is important to know which form is stable and effective and how to change them from an inefficient form to other efficient forms. The H-form crystal was now considered more suitable for use in medicines than the B-form[4]. The S-Form is a new crystal form of nateglinide and its stability is the best among the three forms[3]. According to the comparing tests(see Table 4), we could see clearly that S-form nateglinide also can significantly reduce the level of blood glucose in high glucohemia mice like the H-Form. Especially after 40 *min* ($P < 0.001$ for S-form, compare with H-form $P < 0.01$), the efficacy is much better, indicating its potential usage as a new oral antidiabetic agent.

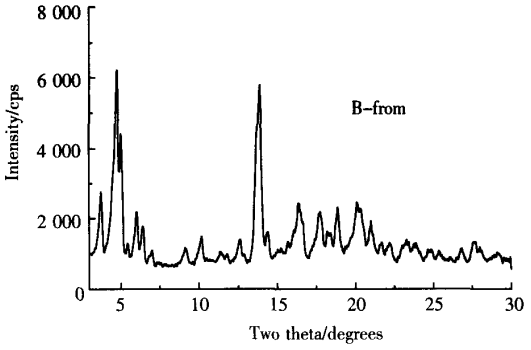

Fig.1 XRD patterns of B-form of nateglinide

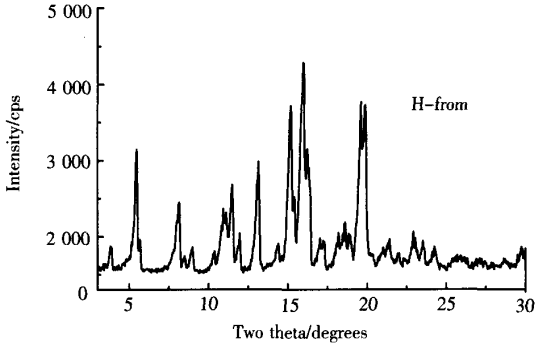

Fig.2 XRD patterns of H-form of nateglinide

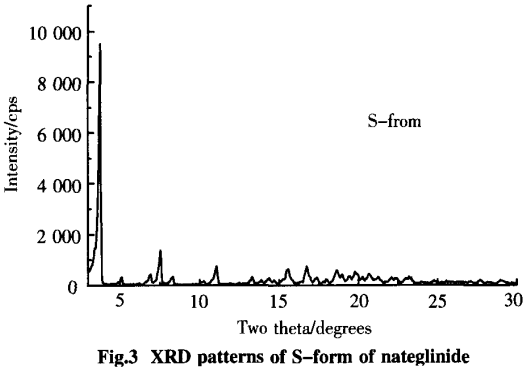

Fig.3 XRD patterns of S-form of nateglinide

Table 4 The results of medicine efficacy test in animals

Items	Mouse Numbers	Dosage/ ($mg \cdot kg^{-1}$)	Values of blood glucose at 20 min/($mmol \cdot L^{-1}$)	Values of blood glucose at 40 min/($mmol \cdot L^{-1}$)	Values of blood glucose at 60 min/($mmol \cdot L^{-1}$)
No. 1(Normal Group)	20		6.059 ± 2.562	6.155 ± 2.738	6.183 ± 2.532
No. 2(Model Group)	20		14.886 ± 3.620	15.627 ± 3.396	15.719 ± 3.231
No. 3(H-form Group)	20	100	11.848 ± 2.778	11.638 ± 2.331 *	11.764 ± 2.611
No. 4(S-form Group)	20	100	12.234 ± 4.441	9.461 ± 2.015 * *	11.612 ± 2.261

compared with model group, * $P < 0.01$, * * $P < 0.001$.

3 Conclusion

Nateglinide has three crystal forms, B-form, H-form and S-form. H-form belongs to the triclinic crystal system with the lattice constant $a = 1.769\,9(7)$ nm, $b = 2.719\,1(8)$ nm, $c = 1.267\,0(6)$ nm, $\alpha = 61.75(3)°$, $\beta = 73.11(6)°$, $\gamma = 66.77(7)°$, $V = 4.895$ nm^3, $Z = 10$. S-form belongs to the orthorhombic crystal system with the lattice constant $a = 2.317\,8(6)$ nm, $b = 2.533\,2(5)$ nm, $c = 0.653\,1(2)$ nm, $\alpha = \beta = \gamma = 90°$, $V = 3.835$ nm^3, $Z = 8$. S-form Nateglinide can significantly reduce the value of blood glucose of administration in high glucohemia mice like the H-form. Especially after 40 min the effect on reducing the level of blood glucose is much better.

[References]

[1] Cao G Y, Hu X, Song Y H. Nonsulfonylurea PGR——nateglinide[J]. Chin J Clin Pharmacol, 2001, 17(3): 231—234.

[2] Li G, Su G Q, Xu Q W, *et al*. A new crystal form of nateglinide[J]. Acta Pharmmaceutica Sinica, 2001, 36(7): 532—534.

[3] Li G, Xu Q W, Mo X Y, *et al*. Study on stability of nateglinide polymorphism[J]. Acta Chimica Sinica, 2003, 61(2): 291—294.

[4] Sumikawa. Crystals of N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine and methods for preparing them[P]. US-Pat: 5488150, 1996-01-30.

[责任编辑:丁蓉]