# Determination of the nateglinide polymorphism structure and the drug effect

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# 那格列奈的多晶结构与药效测定

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摘要: 目的 测定那格列奈的多晶结构和药效, 给出晶格参数和动物试验数据。方法 X射线粉末衍射法测定物相和晶格参数; 腹腔注射葡萄糖致使小鼠形成高血糖血症。小鼠分成对照组、模型组、H晶型治疗组和 S晶型治疗组。测定给药 20, 40和 60 min后小鼠的血糖值。结果 那格列奈有 3种晶型, B-, H和 S型结构。H晶型为三斜晶系, 晶格参数 $a$ = 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, 晶格参数 $a$ = 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。小鼠模拟降糖试验表明: 与 H晶型一样, S晶型也能有效地降低血糖值, 特别是用药 40 min后效果更加明显。结论 H晶型为三斜晶系, S晶型为正交晶系, 用 S晶型给药 40 min后效果更加明显。

关键词: 那格列奈; 多晶型; X射线粉末衍射; 晶格参数; 药效

Type II diabetes is the most common form of diabetes and it occurs in human being of all races and ages. Nateglinide is a novel oral hypoglycemic agent for the therapy 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 weak 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 the three crystal forms of nateglinide was also determined[3].

In this article, the crystal structures of H-form and S-form nateglinide were determined, and their drug effect was also tested and compared.

## 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 in B- or H-form could be changed to S-form in pure methanol. When H-form was heated to melt, and cooled to room temperature slowly, S-form could be obtained, too. None of them can be dissolved in water.

The X-ray powder diffraction patterns of three

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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°• $min^{-1}$ 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 alphal 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, separately.

Eighty ICR (Institnte of Cancer Research) mice (about 22 - 25 g, male/female = 1∶1, manufacture licence number: SCXK, Jiangsu, 2002-0031), were divided into four groups, and used to test and compare the drug effect of H-form and S-form nateglinide. No. 1 was normal group, No. 2 was model group, and No. 3 and No. 4 were fed with H-form and S-form nateglinide, respectively. The glucose test kit was from Shanghai Rongsheng Co. (No. 20020102). The hyperglycemic mice induced by injecting (ip) 2 g• $kg^{-1}$ glucose were administrated with S-form or H-form nateglinide 100 mg• $kg^{-1}$. Then the values of blood glucose were determined after 20, 40 and 60 min of administration, separately.

## Results and discussion

X-ray powder diffraction analysis indicated that there are three kinds of crystal structures existed in nateglinide and their patterns are shown in Figure 1. 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°. This result showed that it has a very strong orientation in the crystal particles.

X-ray powder diffraction analysis also 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, α = β = γ = 90°, $V$ = 3.835 $nm^3$, $Z$ = 8.

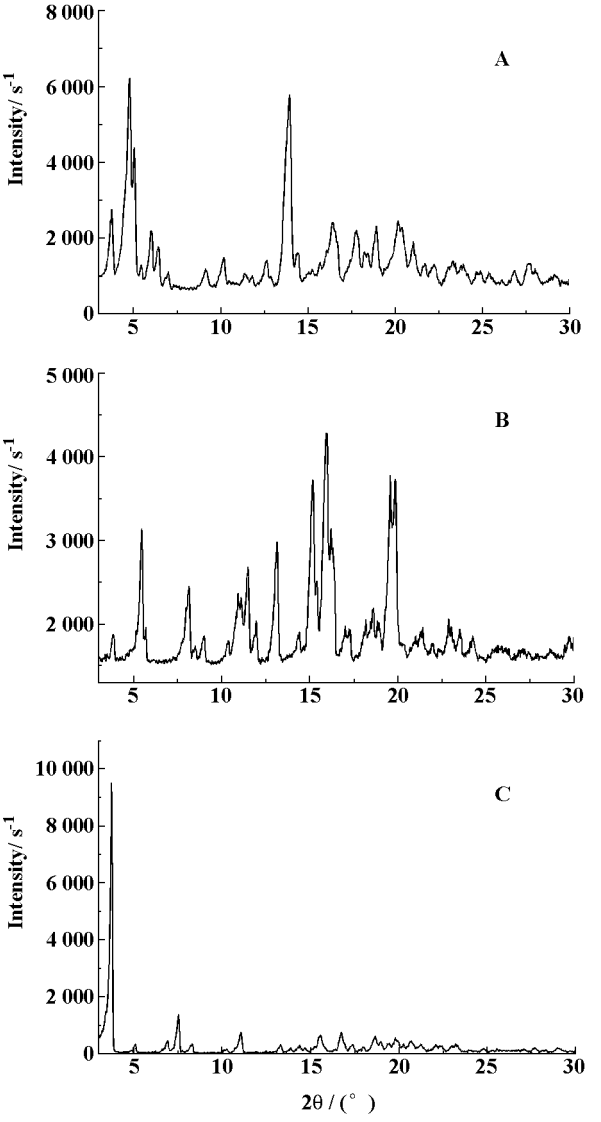


**Figure 1** XRD patterns of B-form (A), H-form (B) and S-form (C) of nateglinide

The drug potency of nateglinide is related with their crystal structure. The 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 different crystal forms 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 to

an 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 comparison tests (Table 1), we could see clearly that S-form nateglinide also can significantly reduce the level of blood glucose in high blood sugar mice like the H-Form. Especially after 40 min ($P < 0.001$ for S-form, compare with H-form $P < 0.01$), the effect is much better. This result indicated that it has potential usage as a new oral antidiabetic agent.

**Table 1** Results of drug effect test in animals

| Group | Dosage /mg·kg$^{-1}$ | Value of blood glucose /mmol·L$^{-1}$ | | |
|---|---|---|---|---|
| | | 20 min | 40 min | 60 min |
| Normal | | 6.1 ±2.6 | 6.2 ±2.7 | 6.2 ±2.5 |
| Model | | 15 ±4 | 16 ±3 | 16 ±3 |
| H-Form | 100 | 11.8 ±2.8 | 11.6 ±2.3* | 11.8 ±2.6 |
| S-Form | 100 | 12 ±4 | 9.5 ±2.0** | 11.6 ±2.3 |

$n = 20$, $\bar{x} \pm s$. * $P < 0.01$, ** $P < 0.001$ *vs* model group

## 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 level of blood glucose of hyperglycemic mice like the H-form. Especially after 40 min the effect is much better.

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