憂鬱症患者尿中新喋呤與生喋呤之分析

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四氫生喋呤（BH_4）是環狀胺基酸水解酶的輔因子，而此水解酶又是合成生物胺(biogenic amines)：5-羥色胺（serotonin)，正腎上腺素（norepinephrine）與多巴胺（dopamine）的速率決定酵素，所以，依"生物胺代謝失調爲憂鬱症病因之一"的假說推論，四氫生喋呤之代謝亦可能與憂鬱症的病理相關。本研究以高效液相層析法（HPLC）測定尿液中四氫生喋呤之代謝物新喋呤(neopterin)與生喋呤（biopterin）之濃度，以探究憂鬱症患者之喋呤類化合物代謝是否與正常人不同。本研究共收集45個正常人與26個有憂鬱症狀之患者的尿液，其中12個患者在憂鬱症狀緩解後再次接受尿液分析。結果顯示尿液中之生喋呤濃度與憂鬱症狀不相同，但憂鬱症患者（441±261 nmol/mmol creatinine）尿中之新喋呤濃度則顯著比正常人（604±318）低（$P<.05$），且在憂鬱症狀緩解後明顯回升（$P<.02$, paired t test, $n=12$），這個現象無法以原始的假設解釋。本文在討論中嚐試探討憂鬱症，免疫功能與新喋呤間的可能關係，並提示進一步之研究方向。（中華精神醫學1991；5：20～8）

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Original Article

Urinary Neopterin and Biopterin Levels in Patients with Depression

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This study was designed to test the hypoghesis that depression is related to tetrahydrobiopterin (BH_4) dysregulation, and to determine if the activity of depression is associated with changes in urinary neopterin levels. Metabolites of BH_4, neopterin and biopterin were determined by reverse-phased high-performance liquid chromatography with fluorometric detection to evaluate the metabolism of BH_4. Urine from 26 patients with active depressive symptoms and 45 normal control subjects were determined. The results showed that the urinary biopterin level in depressed patients (635 ± 281 nmol/mmol creatinine) was similar to that in controls (614 ± 267) ($p < 0.05$), while the neopterin level was significantly lower in acutely depressed patients(441 ± 261) compared with controls (604 ± 318); ($p < 0.05$). Twelve of the depressed patients also had their urine analyzed when they returned to a remission phase. The urinary neopterin level tended to increase along with the improvement of depressive symptoms (360 ± 203 vs. 576 ± 181, paired t test, $p < 0.02$). The signigficance of changes in the urinary neopterin level in depression deserves further exploration.

Key words: depression, tetrahydrobiopterin, neopterin, biopterin
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Introduction

Tetrahydrobiopterin (BH_4) metabolism in patients with depression has beenstudied by various groups of investigators in an attempt to identify its relationship with depression. BH4 is a cofactor of the aromatic amino acid hydroxylases which catlayze the initial and rate-limiting reactions in the synthesis of biogenic amines-serotonin, norepinephrine and dopamine[1,2]. Thus BH4 is of considerable importance in regulating synthesis of these biogenic amines[3]which are proposed as imporatnt neurotransmitters in the pathogenesis of affective disorders [4,5]. After reports that $BH_4$was effective in the treatment of some depressives[6,7] and that the BH_4 concentration was reduced in the post-mortem brain samples of depressive patients[8], the issue of the

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relationship between BH_4 metabolism and depression has been raised by many researchers.

Because BH_4 is unstable, readily oxidized and requires an electrochemical detector, measuring its colsely related metabolites, neopterin and biopterin, was the method of choice for screening and differential diagnosis of defects in BH_4 metabolism. Neopterin is a derivative of dihydroneopterin triphosphate (NH_2P_3), a precursor of BH_4, and biopterin is the fully oxidized form of BH_4. Fukushima & Nixon[9] used a reverse-phase HPLC technique to separate neopterin and biopterin from other unconjugated pterins. This method was later modified and first applied in diagnosing variants of phenylalanin ketonuria (PKU)[10,11]. Garbutt et al[12] was the first group to test the relationship between psychiatric disorders and the biopterin level in cerebrospinal fluid (CSF). No significant difference in the CSF biopterin level between schizophrenics and controls was found in that study. Latter, Duch et al[13,14] and Garbutt et al[12] reported an elevation in the urinary biopterin level of patients with depression; Hashimoto et al[15-17] reported that the plasma level of biopterin was elevated, but BH_4 was reduced in patients in a depressive phase compared with normal controls. The study of Coppen et al[18] found that female patieints with an affective disoder had a lower urinary biopterin level than female controls. Such inconsistency makes it difficult to document the role BH_4 in depression, and further exploration and validation are needed. On the other hand, neopterin, in an independent role, has been reproted to be related to the activities of various diseases such as AIDS[19], tuberculosis[20], malaria, graft-versus host disease[21], Crohn's disease[22], ulcerative colitis [23], rheumatoid arthritis [24], sepsis [25], various malignancies [26], and immune mediated liver disease [27]. This work documents a positive correlation between neopterin concentrations and other indices of disease activity, and led to the proposal that neopterin assays could be used more widely in clinical pracitce. The relationship between depression and neopterin, as an independent marker, has not yet been seriously examined.

In order to elucidate the role of BH_4 and neopterin in depression, we designed this study to compare the level of urinary neopterin and biopterin in patients with depression and in control subjects.

Subjects and Methods

Twenty-six patients (age: 52 ± 15) with active depression according to the diagnostic criteria for a major depressive episode of DSM-IIIR (the revised third edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders) were studied. Five of them had manic histories; six were combined with anxiety disorders. Twelve of the patients had their urine analyzed in both the activve depressive and remission phases. In the context of this paper, the phase was delineated as a score, rated with a Hamilton Depression Rating Scale (HDRS)[28], greater than or equal to 17 for active depression and less than equal to 10 for remission. A rating for each patient was completed by two staff psychiatrists within 4 hours after urine had been collected. At the time of urine sample collection, 14 of the 26 patients had been on the heterocyclic antidepressants, 6 on hypnotic benzodizepines, 3 on herbal medicines, and 3 were free from any medication. Forty-five healthy subjects (age 46 ± 18) from the community were

used as controls. None of them had a history of a psychiatric disorder. All subjects in this study had been free from any significant medical illness, especially immunological disturbances, for at least 3 months. Urine samples were collected in the afternoon from 2 to 5 p.m. and then stored in −20 ℃ until assayed. In pretreatment, urine samples were deproteinized with 40% triacetic acid (TCA). NH_2 was oxidized to neopterin and BH_4 and dihydrobiopterin (BH_2) to biopterin with manganese dioxide (MnO_2). Urinary levels of total neopterin (NH_2 + neopterin) and total biopterin (BH_4 + BH_2 + biopterin) were analyzed by HPLC using a Spherisorb−S5 ODS_2 column (0.46 x 25) and a Lichrosorb RP−18 (Merk) precolumn and were measured with a fluorometric detector (Jasco 820−FP) using an excitation wave length of 350 nm and an emission wave length of 450 nm. The mobile phase was 3% methanol isocratic solvent. The analyzing temperature was 45℃ and the flow rate was 1.0 ml/miinute. Calculation of neopterin and biopterin levels in urine samples was performed by comparing peak heights with those of external standards, which were obtained from Schircks Laboratories (Jona, Switzerland). The coefficiency of variance (C.V.) for within run, run to run and the recovery rate were 5.3%, 7.8% and 87% for the neopterin levels and 5.9%, 7.0% and 85% for the biopterin levels, respectiverly. Urinary creatinine levels were measured by the Beckman Creatinine Analyzer II with a colorimetric Jaffe reaction, the C.V. for within run and run to run were 2.1% and 2.8%, respectively. Each sample was duplicated. Allowances for variations in urine concentrations, levels of neopterin and biopterin in this paper are expressed as nmol/mmol creatinine.

Results

Urinary pterin levels (mean ± SD nmol/mmol creatinine) in the whole group of the depressed patients and the control subjects were compared. The urinary biopterin level for the depressed group (635 ± 281) was not significantly different from the control group (614 ± 267) (t=0.30, P=0.763); but the urinary neopterin levels were lower in depressed patients (441 ± 261) than in the control subjects (604 ± 318) (t=2.21, P=0.03) (Talbe 1). In order to explore the significance of the decreased urinary neopterin levels in depressin, 12 of the depressed patients were also examined in their remission phases. The mean urinary neopterin level was significantly higher in patients in the remission phase (567 ± 181) than in the actively depressive phase (360 ± 203) (t=2.75, P=0.019, paired t test); whereas the biopterin level did not change significantly with improvements in depressive symptoms ($P>0.1$) (Table 2).

The scores for HDRS and values for the urinary pterin level were analyzed by a simple correlation test. Neither the urinary neopterin nor the biopterin levels were significantly correlated with the HDRS scores in patients with depression. The effect of heterocyclic antidepressants on urinary pterin excretion was examined. Antidepressant−treated patients and antidepressant−free patients excreted similar levels of neopterin (t=0.01, P=0.993) and biopterin (t=0.30, P=0.776) (Table 3). Urinary pterin levels were not correlated with age.

Discussion

Our results showed a significant difference in the urinary neopterin level, but

Table 1. Urinary pterin levels in depressed patients and control subjects

	Depression	Control
No. (male/feamle)	26 (14/12)	45 (16/29)
Age	52±15	46±18
HDRS score	21±4	.....
Neopterin	441±261*	604±318
Biopterin	635±281	614±267

Units of pterins are nmol/mmol creatinine;
* P=0.03 from the control group.

Table 2. Urinary pterin levels in active depressive and remission phase

	Active depression	Depression in remission
No. (male/feamle)	12 (7/5)	12 (7/5)
Age	53±14	53±14
HDRS score	21±4	7±3
Neopterin	360±203*	567±181
Biopterin	664±246	647±162

Units of pterins are nmol/mmol creatinine;
* P=0.019 from the control group.

Table 3. Urinary pterin levels in antidepressant-treated and antidepressant-free patients

	Antidepressant-treated	Antidepressant-free
No.	14	12
Neopterin	441±282	440±248
Biopterin	650±320	616±242

Units of pterins are nmol/mmol creatinine;
* P=0.99 for neopterin & 0.77 for biopterin by t test.

not the biopterin level, between depressed and control subjects. The exisiting studies demonstrated a marked inconsistency for the relationship between the biopterin level and depression. Thus, the hypothesis that depression is related to a distrubance in BH_4 metabolism is unfavored. In fact, pterins do not only exist in neural tissue, they are also found in peripheral tissues such as the liver, spleen, and adrenal cortex[13]. The central nervous system contributes insignificant amounts of biopterin to the urine [30]. So, BH_4 metabolis in the brain, the center of mood control, cannot be simply reflected by the urinary level of biopterin. A de crease in the neopterin level was not associated with a decrease in the biopterin level, indicating that role of neopterin is not only as the precursor of BH_4. Neopterin has been demonstrated to be associated with the activities of a variety of diseases, and measurement of the neopterin level has been applied in clinical use. For example, the neopterin level was monitored daily in several European transplant patients, because an increasing neopterin level indicates immunological complications in allograft recipients[31,21]. The urinary neopterin level was used as an adjunctive marker in evaluating the preoperational condition of patients with colorectal cancer[32]. The relationship between depression and neopterin as an independent factor has never been discussed in a published paper. The present results indicate a possible linkage between neopterin and depression and deserve further validation and exploration. The significance of the neopterin change has been demonstrated to be related to immunological activity[33,34]. On the other hand, depression has been shown to be characterized by activation of the hypothalamic–pituitary–adrenal (HPA) axis with uninhibited steroid output[35], which may suppress the immune function. Furthermore, several controlled studies have supported the hypothesis that a substantial percentage of depressed patients show a suppressed immune response on in vitro measure of cellular immunity[36–38]. Along this line, we raise a preliminary question: Is alteration in urinary neopterin in patients with depression mediated through an immunological reaction? Further work involving simultaneous measurement of the urinary neopterin level, HPA axis functioning, and various immunity indices such as lymphocyte responses to mitogen stimulation, natural killer cell acitvity, and measures of the T cell subpopulation, is required to clarify this question. For practical and ethical considerations, patients who were suffering from depressive symptoms were not asked to remain drug–free for a period of time, instead, they were divided into antidepressant–treated and antidepressant– free groups according to the medication, regardless of whether the medication was benzodiazepan or an herbal medicine, that the patient was on when he or she entered this study. The effect of antidepressants on pterin excretion was not significant. This result was in accrod with that of Garbutt et al [12]. Although Shintaku et al[29] reported that the urinary neopterin concentration was higher in neonates than in children and adults, our results, which were all from adults, did not show any correlation between age and urinary pte - rin levels.

Several important and fundamental porblems in this study have to be mentioned. First, although the patients in this study all fit the diagnostic criteria of DSM–IIIR for a major depressive episode and had an HDRS score of greater than 17, they did come from several diagnostic

categories, including bipolar disorder, major depression and depression secondary to anxiety disorder. Accordinlgy, the present results can only account for a heterogeneous group of patients with major depressive symptoms, regardless of the underlying cause, rather than a homogeneous group with endogenous depression or a subtype of depression. Second, despite the existence of a statistically significant differene, the lack of correlation between HDRS and the urinary neopterin level is difficult to explain, and with the present data, the urinary neopterin level was neither sensitive nor specific enough as a biological indicator to differentiate individuals with or without depression. Third, the results of previous studies [12,13,18] which have determined urinary neopterin levels in patients with depression are different from this study, although they are also discordant. A more sophisticatedly designed study, including a more homogeneous grouping of depressed patients, with a larger sample size is needed to verify our findings. Fourth, although pterin excretion was reported to be constant throughout a 24-hour period [30], and the original pterin levels were divided by the creatinine level to eliminate the distrubing effects of different urine concentrations, the variance raised by the procedure of dividing may have exceeded a tolerable level. Collecting 24-hour urine or multiple measures at different times could be a choice to reduce such errors. Fifth, since the neopterin level rose in response to various common infections, subjects with subclinical infection may have been entered this study and created false-elevated neopterin levels, especially for the control subjects who were not followed up after urine sample collection. In addition to clinical evaluation, virus titer determination such as ELISA or a complement fixation test could be taken as an adjunctive tool to rule out possible subclinical infections.

In summary, the present results do not support the hypothesis that biopterin deficiency plays a role in the pathogenesis of depression. The finding that the urinary neopterin level changes with the activation and remission of depression indicates a possible linkage between neopterin and depression. This finding needs further validation and this significance deserves exploration. The preliminary inference of the immunological mediation of neopterin in depression will direct our subsequent investigations into the biological pathogenseis of depression.

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