

In animal models, immune activation is often difficult to assess because of the limited availability of specific assays to detect cytokine activities. In human monocytes/macrophages, interferon- γ induces increased production of neopterin and an enhanced activity of indoleamine 2,3-dioxygenase, which degrades tryptophan via the kynurenine pathway. Therefore, monitoring of neopterin concentrations and of tryptophan degradation can serve to detect the extent of T helper cell 1-type immune activation during cellular immune response in humans. In a porcine model of cardiac arrest, we examined the potential use of neopterin measurements and determination of the tryptophan degradation rate as a means of estimating the extent of immune activation. Urinary neopterin concentrations were measured with high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA) (BRAHMS Diagnostica, Berlin, Germany). Serum and plasma tryptophan and kynurenine concentrations were also determined using HPLC. Serum and urine neopterin concentrations were not detectable with HPLC in these specimens, whereas RIA gave weakly (presumably false) positive results. The mean serum tryptophan concentration was $39.0 \pm 6.2 \mu\text{mol/l}$, and the mean kynurenine concentration was $0.85 \pm 0.33 \mu\text{mol/l}$. The average kynurenine-per-tryptophan quotient in serum was $21.7 \pm 8.4 \text{ nmol}/\mu\text{mol}$, and that in plasma was $20.7 \pm 9.5 \text{ nmol}/\mu\text{mol}$ ($n = 7$), which corresponds well to normal values in humans. This study provides preliminary data to support the monitoring of tryptophan degradation but not neopterin concentrations as a potential means of detecting immune activation in a porcine model. The kynurenine-per-tryptophan quotient may serve as a short-term measurement of immune activation and hence permit an estimate of the extent of immune activation.

key words: Tryptophan, Kynurenine, Indoleamine (2,3)-dioxygenase, Neopterin, Animal model

Monitoring of immune activation using biochemical changes in a porcine model of cardiac arrest

Anton Amann^{1,CA}, Bernhard Widner², Josef Rieder¹, Herwig Antretter³, Georg Hoffmann⁴, Viktoria Mayr¹, Hans-Ulrich Strohmenger¹ and Dietmar Fuchs²

¹Department of Anesthesiology and General Intensive Care, University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria; ²Department of Medical Chemistry and Biochemistry, University of Innsbruck, and Ludwig Boltzmann Institute of AIDS Research, Innsbruck, Austria; ³Department of Cardiac Surgery, University of Innsbruck, Austria; and ⁴Department of Physiology, University of Bonn, Bonn, Germany

CA Corresponding author

Tel: +43 512 504 463

Fax: +43 512 504 4683

E-mail: anton.amann@uibk.ac.at

Introduction

In animal models, immune activation is often difficult to assess because of the limited availability of (species)-specific assays to detect cytokine activities. Moreover, the biologic half-life of cytokines is low, and they may rapidly bind to target cells or be inactivated by, for example, soluble receptors, thereby escaping measurement. In humans, measurement of neopterin in serum or urine has been established as a sensitive marker of cellular, i.e. T helper cell 1-type, immune response.¹ Neopterin is a stable, low-molecular mass compound (253 Da) produced in increased concentrations by interferon (IFN)- γ -stimulated human and primate monocytes/macrophages. In these cells, neopterin is produced at the expense of biopterin, because of a relative

deficiency of 6-pyruvoyltetrahydropterin synthase (PTPS), the enzyme responsible for biosynthesis of 5,6,7,8-tetrahydrobiopterin. In other cells, and also in monocytes/macrophages of other species, PTPS activity is constitutively high, and no or very low concentrations of neopterin can be detected.² The clinical use of neopterin determination ranges from surveillance of allograft recipients for early detection of immunological complications in order to predict prognosis, to control of therapy in malignant diseases and HIV infection.^{1,3}

IFN- γ also stimulates indoleamine 2,3-dioxygenase (IDO), an enzyme degrading free tryptophan in a broad variety of cells including macrophages to form *N*-formylkynurenine, which is subsequently deformed to kynurenine.⁴ Thus, the simultaneous determination of kynurenine and tryptophan in serum and

calculation of the kynurenine-per-tryptophan quotient provide an estimate of IDO activity, and hence of endogenous formation of IFN- γ .^{5,6} In conditions of cellular immune activation in humans, a correlation exists between serum neopterin concentrations and the kynurenine-per-tryptophan quotient.^{5,7}

In animal model systems, except simian, neopterin measurements have not been considered an appropriate means of assessing immune activation status because previous trials to determine neopterin concentrations in animals mostly revealed concentrations below the detection limit of available assay systems.⁸ With the improvement of analytical methods over the past 15 years, however, the sensitivity of assays to detect neopterin in body fluids has been significantly enhanced.

In a porcine model of cardiac arrest, we re-examined the potential use of neopterin measurements and the determination of the tryptophan degradation rate with a view to estimating the extent of immune activation.

Methods

Animals

This study was performed according to Utstein-style guidelines⁹ in seven healthy, 18- to 20-week-old swine weighing 61 ± 8 kg.

Surgical preparation and experiment protocol

Instrumentation, pre-medication and anesthesia were performed as described elsewhere.¹⁰⁻¹² In brief, a saline-filled catheter was advanced into the abdominal aorta to measure aortic blood pressure and take blood samples. Fifteen minutes before cardiac arrest, 5000 U of heparin were administered intravenously to prevent intracardiac clot formation. Instead of external manual chest compression, a heart-lung machine was used to control the blood flow in this reanimation model. Three episodes of cardiac arrest were induced by a 50-Hz, 60-V alternating current applied via two subcutaneous needle electrodes, and ventilation was discontinued at that point. After a period of cardiac arrest (4 min in episode 1; 8 min in episodes 2 and 3), a reduced blood flow (10% of normal blood flow during episode 1; 20% of normal blood flow during episodes 2 and 3) was supplied by the heart-lung machine. The total duration of the episodes was 22 min (episode 1) and 10-14 min (episodes 2 and 3). After each episode, an attempt was made to defibrillate the pigs. If electric defibrillation was not successful, sinus rhythm was restored by providing 60% blood flow via the heart-lung machine. Between the cardiac arrest episodes, the pigs were given a recovery phase of 1 h. The total duration of the experiment was 5 h.

Measurements

Blood samples were collected from a catheter in the arteria femoralis immediately after instrumentation to obtain baseline values. Further specimens were drawn before starting each episode and at the end of the experiment before necropsy. Blood samples were placed in serum monovettes or heparinized blood monovettes and centrifuged at $1500 \times g$ for 10 min at 4°C. The supernatant, i.e. plasma or serum, was stored at -20°C until measurement. Urine was collected immediately after instrumentation and stored at 4°C for a maximum of 2 days.

Urinary neopterin concentrations were measured with high-performance liquid chromatography (HPLC)¹³ and radioimmunoassay (RIA), permitting detection of 0.2 nmol/l neopterin (BRAHMS Diagnostica, Berlin, Germany). Concentrations were divided by urinary creatinine concentrations, which were simultaneously determined using HPLC, to account for differences in urine densities. Serum and plasma tryptophan and kynurenine concentrations were determined with HPLC.⁶ The method was modified slightly: a LiChroCARTTM 55-4 RP18 column (MERCK, Darmstadt, Germany) was used, which reduced analysis time to 7 min while ensuring sufficient analytical performance.

Statistics

The data are reported as Mean \pm SD. Comparison of different groups is performed using analysis of variance. We considered two-tailed $p < 0.05$ statistically significant.

Admission

This project was approved by the Austrian Federal Animal Investigation Committee, and the pigs were managed in accordance with the American Physiological Society and institutional guidelines.

Results

Mean urinary neopterin concentration (\pm SD) was 9.5 ± 5.0 nmol/l (range, 3.4 - 17.1 nmol/l; $n = 7$) as measured by RIA. However, the results were not confirmed by HPLC, having a detection limit of 1.0 nmol/l. Neopterin concentrations in all the urine specimens were below this limit. Serum neopterin concentrations were < 0.2 nmol/l in all serum/plasma specimens and hence below the detection limit of the RIA. The mean serum tryptophan concentration was 39.0 ± 6.2 μ mol/l, and the mean kynurenine concentration was 0.85 ± 0.33 μ mol/l. The average kynurenine-per-tryptophan quotient in serum was 21.7 ± 8.4 nmol/ μ mol and that in plasma was 20.7 ± 9.5 nmol/ μ mol ($n = 7$). The kynurenine-per-trypto-

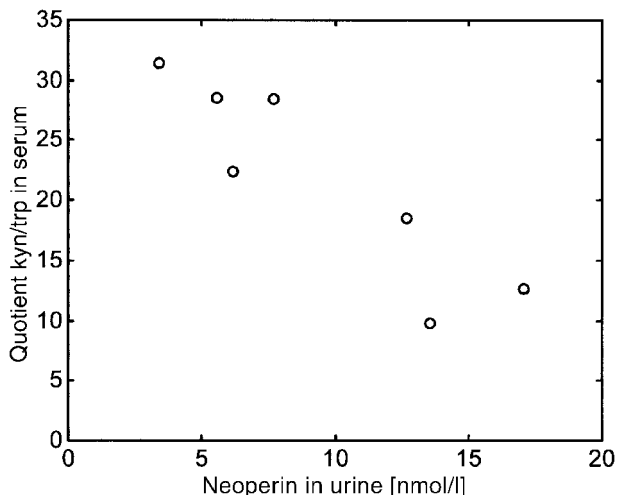


FIG. 1. Kynurenine-per-tryptophan (kyn/trp) quotients measured in serum versus concentration of neopterin in urine (baseline values).

phan quotients in serum and plasma were linearly correlated ($r = 0.94$; $p = 0.0016$; $n = 7$), and there existed an inverse relationship between the kynurenine-per-tryptophan quotients and the urine neopterin concentrations measured by RIA ($r = -0.819$, $p < 0.001$; Fig. 1).

Fig. 2 shows the development of the kynurenine-per-tryptophan quotient in serum between *baseline* values and the values obtained at the *end of the reanimation* experiment. In female pigs, the quotient increased ($p < 0.05$; $n = 4$), whereas no such difference was seen in male pigs ($p = 0.66$; $n = 3$).

Discussion

Increased neopterin concentrations and an enhanced tryptophan degradation rate reflect the extent of T helper cell 1-type immune activation during cellular immune response in humans. This study provides

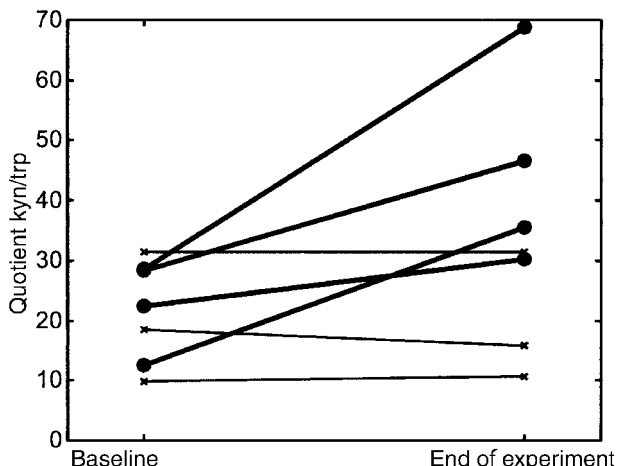


FIG. 2. Kynurenine-per-tryptophan (kyn/trp) quotients measured in serum for baseline and at the end of the reanimation experiment (male (x) and female (o) animals).

preliminary data in support of the potential use of tryptophan degradation but not neopterin concentrations as immune activation markers in a porcine model. Urinary concentrations of neopterin in the non-primate animals were detected by RIA, and the baseline magnitude of measured neopterin concentrations lies well within a range accessible with standard analytical methods like immunoassays or HPLC. However, HPLC measurements could not confirm the results obtained with RIA. An analytical uncertainty is the possible cross-reaction of the assay antibody with dihydroforms of neopterin and with biopterin derivatives,¹⁴ other pteridines that are excreted via the kidneys in considerable amounts. It thus seems very likely that the neopterin concentrations measured by means of RIA in the porcine model are biased by a possible cross-reaction. Further studies are necessary to clarify this issue. In addition, an inverse relationship was found between the kynurenine-per-tryptophan quotients and the neopterin concentrations measured by RIA. Previous studies conducted in humans commonly showed a positive relationship,⁵⁻⁷ which agrees with the background common for tryptophan degradation and neopterin production, both of which are induced by cytokine IFN- γ . Thus, the neopterin concentrations detected with RIA are highly questionable. Serum or plasma neopterin concentrations were all < 0.2 nM, which is still below the detection limit of the available immunoassay.

More relevant information about immune activation status was gained from the tryptophan degradation rate in serum. Baseline values of the kynurenine-per-tryptophan quotient in pigs approximately correspond to values in humans,^{5,6} and serum or plasma specimens are equally suited for HPLC measurement of tryptophan and kynurenine. After the experiment, an increase was seen in the quotient, which also lies in the range of human values during infectious or inflammatory disorders.⁵ The increased degradation of tryptophan during the reanimation experiment was apparent only in female pigs, which could be attributed to the small number of animals tested. On the contrary, hormonal differences could also play a role; for example, estradiol was found to affect tryptophan metabolism in rat liver.¹⁵

The kynurenine-per-tryptophan quotient may enable short-term measurement of immune activation and thus an estimate of the extent of immune activation in porcine animal models (e.g. ischemia/reperfusion experiments, cardiac arrest, hypovolemia or endotoxic shock syndrome). When tryptophan catabolism in pigs is analogously controlled like in humans, high values can be expected in, for example, an endotoxic shock model.

ACKNOWLEDGMENTS. This work was supported by the Austrian 'Fonds zur Förderung der wissenschaftlichen Forschung' (project 14154 Med), by the Austrian National Bank (project 7276), by the Austrian Heart Foundation (project 98/05), and by the Austrian Federal Ministry of Social Affairs.

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Received 3 July 2001

Accepted 6 September 2001