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Determination of renal clearance of neopterin by a pharmacokinetic approach

Willibald Estelberger[a], Günter Weiss[b], Walter Petek[c], Benno Paletta[a], Helmut Wachter[b] and Gilbert Reibnegger[b]

[a]*Institut für Medizinische Chemie, Universität Graz, Austria,* [b]*Institut für Medizinische Chemie und Biochemie, Universität Innsbruck, Austria* and *Institut für Medizinische Biochemie, Universität Graz, Austria*

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Pharmacokinetic modelling was used to determine the glomerular filtration rate and tubular secretion of neopterin, a marker for cellular immune activation. The method involves parameter identification employing the transient venous plasma concentration profiles of marker substances. By combined i.v. injection of neopterin and inulin which is excreted exclusively via glomerular filtration, neopterin was shown to be excreted in addition to glomerular filtration, by tubular secretion: clearance of inulin, 112 (S.D. 2.2) ml/liter; clearance of neopterin, 499 (S.D. 79.7) ml/min. A pilot experiment using in addition *p*-amino hippuric acid suggests that neopterin and *p*-amino hippuric acid may employ the same carrier system for tubular secretion.

Neopterin, Renal clearance, Glomerular filtration; Tubular secretion; Pharmacokinetics; Mathematical modelling

1. INTRODUCTION

During the past decade, interest in the clinical application of neopterin has greatly increased. Neopterin belongs to the class of pteridines which are pyrazino-[2,3-*d*]-pyrimidine compounds biosynthesized from guanosine triphosphate. Chemically, neopterin is 2-amino-3,4-dihydro-4-oxo-6-(1′*S*,2′*R*)-1′,2′,3′-trihydroxypropyl-pterin (Fig. 1). It is biosynthesized in relatively large amounts by human macrophages upon stimulation with interferon-γ [1], and measurement of its concentration in various body fluids has been demonstrated to be of clinical interest in various diseases; elevated neopterin levels are a sensitive marker for the activation of cellular immune system [2,3].

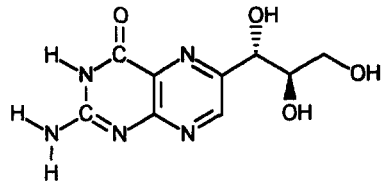
After its interferon-γ-mediated biosynthesis, neopterin is metabolically stable, and is excreted via the kidneys. The concentration of neopterin is usually determined in blood (serum, plasma) or urine, and it depends on renal function. Patients with impaired renal function show higher neopterin levels in their circulation. In urine, due to normal variability of urine density, it is usual practice to relate neopterin concentrations to simultaneously determined urinary creatinine levels. There are experiments suggesting that neopterin might be excreted via tubular secretion in addition to glomerular filtration [4,5], but precise knowledge on the excretion kinetics of neopterin is lacking.

Correspondence address: G. Reibnegger, Institut für Medizinische Chemie und Biochemie, Fritz Pregl Straße 3, A-6020 Innsbruck, Austria. Fax: (43) 512 507 2279.

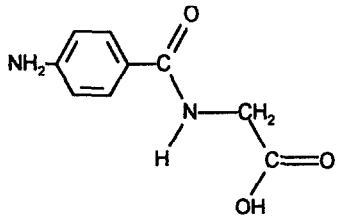
On the other hand, such knowledge is required for proper interpretation of neopterin concentrations in patients who in addition to an immunologically mediated disease suffer from renal damage. For example, neopterin measurement is widely used to monitor recipients of renal allografts [6,7], and one has to be careful in differentiating neopterin elevations due to immune activation from mere excretion problems.

Measurement of creatinine clearance is commonly used for determination of renal function but the methods has serious drawbacks, and various methods have been suggested to oversome these problems, e.g., by medication [8]. We recently described an alternative way which employs mathematical modelling of temporal venous plasma concentration profiles of marker substances [9,10]. This approach enables one to obtain precise estimates of the rate constants determining transport processes of a substance between various body compartments and elimination kinetics via the kidneys. Linear as well as non-linear kinetics can be modelled by this approach. In contrast to more conventional techniques, the method is based on repeated measurements of the time-dependent concentration of marker or test substances in venous blood after venous bolus infusion, and neither measurements in urine nor collection of 24-h urine is required. Monte-Carlo simulation is then applied to obtain estimation errors of the model parameters determined by this technique in individual subjects.

We use this approach here for determination of the detailed elimination kinetics of exogenously administered neopterin. In addition to investigating, with the



Neopterin



p-Amino hippuric acid

Fig. 1. Chemical structure of neopterin and *p*-amino hippuric acid.

same model, the elimination of two different doses of neopterin, we also study the effect of simultaneously applying a bolus injection of neopterin together with *p*-amino hippuric acid (Fig. 1) in order to investigate whether both substances compete for renal transport.

2. MATERIAL AND METHODS

2.1. *Subject*

The experiments were all done using one of the authors (G.R.; male, 37 years, body length 178 cm, body weight 70 kg) as volunteer. The subject was clinically healthy at the time of the experiments, and did not receive any medication. Nutrition was normal diet.

2.2. *Chemicals*

Synthetic neopterin was obtained from Dr. B. Schircks, Jona, Switzerland. This product was previously shown to contain no measurable endotoxins [11]. Furthermore, inulin (INUTEST, Laevosan, Linz, Austria) which is conveniently measured by a fully enzymatic method, *p*-amino hippuric acid (Nephrotest, Biologische Arbeitsgemeinschaft, Lich/Hessen, Germany) and isotonic sodium chloride solution (Heilmittelwerke Wien, Vienna, Austria) were used.

2.3. *Study design*

Three separate experiments were performed, with more than one month interval in between. For each experiment, 10 ml of the inulin solution containing 2,500 mg were used. In addition, 10 ml of freshly dissolved neopterin in sodium chloride solution were used. In experiment no. 1 a low neopterin dose was injected, in experiment no. 2 a high amount was used. In the third experiment, 10 ml isotonic saline containing 2,200 mg of the sodium salt of *p*-amino hippuric acid were additionally applied.

The subject, after acquiring a lying position for 30 min before the experiment, received the substances by bolus injection into the right cubital vein during the initial five min of each experiment, and blood samples were then drawn from an intravenous catheter placed in the left cubital vein in 5-min intervals during the subsequent two h. An extra blood sample was drawn immediately before injection of test substances in order to obtain a base line value of endogenous neopterin.

2.4. *Pharmacokinetics*

The model used is the basic model of pharmacokinetics [12] which was used either in the linear version, or was adapted in order to allow modelling of non-linear effects resulting from capacity-limited tubular secretion when using high doses of markers which are transported by the tubular anion carrier system, such as *p*-amino hippuric acid. This is of relevance due to the limited number of carriers involved in the secretory process. Secretion is furthermore inhibitable by competition of many endogenous and exogenous substances employing the same carrier system as transport mechanism.

The model equations employed for mathematical modelling are described fully elsewhere [9,10].

For inulin which is known to be excreted by glomerular filtration only, and for neopterin of which rather low absolute amounts were used, the full model was simplified by assuming that the simple linear two-compartment model is appropriate for which analytic solutions of the differential equations are well-known. For *p*-amino hippuric acid, however, a high dosis was used, and the full non-linear model was employed in order to deal correctly with the non-linear behavior.

For the numerical integration of the non-linear system, the fourth-order Runge-Kutta technique was used. Fitting of this model to the measured concentration data was done by a method of direct search employing steepest-descent technique to locate the minimum of the error function, defined by the residuals of the fit.

Measured data are subject to different kinds of errors. Thus, the fitted model parameters are associated with estimation errors. In order to gain estimates for these parameter errors, after fitting the original data repeated runs of the parameter identification procedure were performed using artificial protocols. These were produced by superposition of Gaussian random numbers on the theoretical trajectory. The random numbers were taken from a hypothetical normal distribution with mean zero and standard deviation defined by the experimentally obtained error function [13] ('Monte-Carlo technique').

3. RESULTS

Table I shows the clearance values for neopterin and inulin obtained from two experiments using different doses of neopterin. As Table I shows, the inulin clearance as a measure of glomerular filtration rate lies in the normal range for healthy individuals. The values estimated for neopterin indicate that in accordance with previous findings, neopterin is excreted both by glomerular filtration and tubular secretion. The clearance values appear to be insensitive to variation of injected neopterin dose.

Table I

Results of mathematical modelling of renal excretion kinetics

	Experiment	
	Low neopterin	High neopterin
Inulin dose (mg)	2,500	2,500
Inulin clearance (ml/min)	111.5 (2.2)*	139.7 (2.3)
Neopterin dose (nmol)	400	3,300
Neopterin clearance (ml/min)	498.8 (79.7)	498.1 (23.1)

*S.D. given in parentheses.

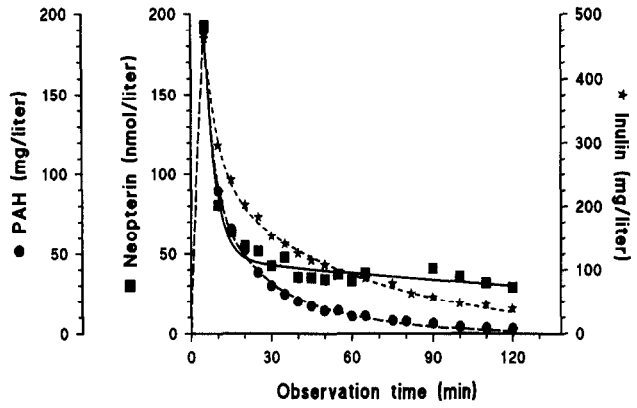


Fig. 2. Temporal venous concentration profiles for inulin, neopterin and *p*-amino hippuric acid after simultaneous i.v. bolus injection. Indicated are the measured concentration values (denoted by symbols) and the fitted model curves (denoted by the lines). Notably, the concentration profiles of inulin and neopterin were treated by a linear model, whereas the elimination kinetics of *p*-amino hippuric acid was modelled by the full non-linear formalism. The renal clearance values estimated from the model were: inulin, 152.8 ml/min (S.D. 2.7); neopterin, 300.4 (65.3); and PAH, 934.7 (59.3).

Fig. 2 shows fitted model trajectories together with measured temporal concentration profiles for the third experiment in which inulin (2,500 mg), neopterin (4,000 nmol) and the sodium salt of *p*-amino hippuric acid (PAH, 2,200 mg) were injected in combination. The clearance for *p*-amino hippuric acid as an index of renal plasma flow lies in a range indicating normal renal function [14]. The markedly lower neopterin clearance value observed in this experiment seems to demonstrate that *p*-amino hippuric acid may significantly depress renal clearance of neopterin, at least if applied in high dosage.

4. DISCUSSION

The precise determination of the elimination kinetics of exogenous and endogenous substances by the kidneys is of considerable interest, both for diagnostic and therapeutic purposes. We investigate here the possibility of applying pharmacokinetic modelling to study the renal handling of neopterin, an endogenous marker substance which has become widely accepted as a sensitive indicator for the activation of cell-mediated immunity in vivo.

The data presented here fully confirm the existence of tubular transport of neopterin which was expected from previous observations [4,5]. Notably, the data were obtained by studying a healthy subject; thus, the result is not biased by the presence of a disease. We are, of course, aware of the fact that a study on one individual only limits the representativity of the results. The reasons for this limitation are the following: since the method used is an invasive one, it is not easy to recruit volunteers. Moreover, at the time when the measurements described here were done, it was generally thought that neopterin is biologically inactive [11]. Meanwhile it was found that neopterin enhances the toxicity mediated by reactive oxygen and chloride species [15]. Therefore, obtaining ethical approvement for investigating further subjects by bolus injection of neopterin would be difficult. On the other hand, although measurements were done on a single individual only, they are likely to be representative since (i) there is a good agreement with expectation from previous, albeit incomplete data [4,5], and (ii), data obtained after bolus injection of two different amounts of neopterin show excellent accordance (see Table I). Moreover, the Monte-Carlo approach used enables statistically correct judgement of the precision of the data obtained from the experiments.

The amounts of neopterin injected in the described experiments may appear low when compared with inulin or *p*-amino hippuric acid. It should be noted in this respect, however, that the concentrations of neopterin which are constitutively found in healthy humans (i.e. in the absence of immunological stimulation), are in the range below 10 nmol/l [2,3], and thus are considerably lower than the peak values observed after bolus injection of the neopterin amounts employed.

A possible application of the pharmacokinetic approach presented here is the investigation of competitive interactions between drugs excreted by the renal tubular system [16]. In the particular example of neopterin, the consideration of such a competitive inhibition of tubular secretion appears to be of immediate relevance, since neopterin concentrations are widely used to aid in the prognosis of patients infected with the human immunodeficiency virus type 1 (HIV-1) [17–22], and the modified nucleosides frequently used for treatment of such patients are known to be eliminated, at least partly, via the same mechanism [23]. Thus, detailed investigation of such putative interactions seems most relevant in order to allow proper interpretation of measured serum neopterin concentrations in such patients.

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