DETERMINATION OF GLOMERULAR FILTRATION RATE IN ADULT HORSES AND DONKEYS BY SINGLE INTRAVENOUS ADMINISTRATION OF IOHEXOL

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15 **Abstract**

16

INTRODUCTION

Glomerular filtration rate (GFR) is considered the best quantitative parameter of overall renal function,[1-3] because it is the sum of the filtration rates of each functioning nephron and represents an index of functional renal mass.[3,4] Thus, an accurate and sensitive measurement GFR is essential for identifying early alterations in renal function to prevent progression of renal disease and damage.[5]

GFR is determined by renal or plasma clearance of an ideal filtration marker, which is freely filtered by the kidney and is neither bound to plasma proteins nor reabsorbed, secreted, or metabolised by the renal tubule and does not affect GFR. The classical reference method for estimation of GFR is the renal clearance of inulin, but this method is not useful in equine routine clinical practice because it requires constant IV infusion of inulin, water loading, and bladder catheterisation to ensure accurate urine collection.[6] The use of inulin in clinical setting has been replaced by that of radiolabelled compounds for measurement of GFR,[1,4,7-13] but facilities for working with radioactive material are required. Iohexol (IOX) is a non-ionic low-osmolarity contrast medium widely used in many species owing to its low toxicity. The renal clearance of IOX is close to the renal clearance of inulin in man thus IOX has been used to estimate GFR in humans[14-17] and in animals.[1,13-14,18-26]

To the authors' knowledge, literature regarding the use of IOX to evaluate GFR in the horse is scarce[24-25] and no data are reported on donkeys. The aims of this study were to estimate GFR using IOX clearance and to evaluate the accuracy of limited sampling models to establish an accurate and clinically suitable GFR test in clinically normal adult horses and donkeys.

MATERIALS AND METHODS

Animals

Eight Standardbred mares (*Equus caballus*) and eight Amiata donkey jennies (*Equus asinus*) were included in the study. Mares belonged to the Department of Veterinary Sciences (Pisa, Italy), aged 8-11 years and weighed 450-560 Kg, while Amiata donkey jennies belonged to the Regional Stud Centre (Pisa, Italy), aged 5-6 years and weighed 350-380 Kg. Approval to conduct this study was obtained from the Ethics Committee on Animal Experimentation of the University of Pisa (D.L. 116/92 - Rettoriale n. 02/1223 del 15/07/2002) and transmitted to the Italian Ministry of Health. Animals have been included in this study if following these criteria: 1) treatment against gastrointestinal parasites and vaccination against equine influenza, tetanus, and equine herpes virus-1 and 4, according to guidelines of the American Association of Equine Practitioners Infectious Disease Committee;[27] 2) animals considered clinically healthy on the basis of a physical examination; 3) blood creatinine[a] and urea[b] concentrations[3] and urine analysis[c] within normal values both for horses and donkeys.[28]

Iohexol administration and blood sampling

Two 14-G catheters[d] were placed into both jugular veins, using aseptic technique. A commercially available IOX formulation[e] was administered IV as a bolus (within 1 minute) at the dose of 75.5 mg/Kg for both equine mares and donkey Jennies, through the right jugular catheter. The exact doses administered were determined from difference between the weights of the syringe and its needle before and after administration. Samples were collected by the catheter positioned into the left jugular vein before (time 0) marker's administration, and at 15, 30, 60, 90, 120, 180, 240, 360 minutes and 12h after. Blood was collected into lithium-heparin test tubes[f] and centrifuged at 3000 rpm within 10 minutes from collection. Plasma was stored in aliquots at –20°C. During the all study period, animals were stabled in 4X4 m boxes, allowed free access to water and hay, in order to

64 better reflex renal function.[5]

65 ***HPLC method***

66 *Chemicals and reagents.* IOX and the internal standard (IS) iopentol were kindly supplied by
67 Nycomed Amersham Sorin[g]. Water was doubly distilled and purified using a Sartorius cellulose
68 acetate filter[h]. High-performance liquid chromatography (HPLC) grade water, dichloromethane and
69 acetonitrile were supplied by LABSCAN[i].

70 *Chromathographic conditions.* The HPLC system consisted of a Series 200 Perkin Elmer gradient
71 Pump coupled to a Series 200 Perkin Elmer variable UV detector which was set at 254 nm. The
72 reversed-phase column was a Sunfire® Waters C_{18} column (5 µm, 250x4.60 mm) connected to a
73 Waters Guard-Pak™ C_{18} pre-column (4 µm)[l]. The column was kept at room temperature.
74 Turbochrome® software was used for data processing. A 20 µl injection was used each time. The
75 mobile phase consisted in acetonitrile-water pH 2.7 (acidified by addition of H_3PO_4 85%). For
76 analysis, the peak area of the major IOX and IS isomer was used because them constituted more
77 than 80% of the combined peak areas and the ratio of both the isomer peaks remained constant at
78 different IOX and IS concentrations under the current analytical condition. All calculations were
79 performed using peak area ratios of the IOX peak to the IS peak (peak area ratio) by the use of
80 Microsoft Excel[m]. IOX was eluted as two isomers at 6.4 and 6.8 min, whereas the IS eluted as two
81 isomers at 10.4 and 11.0 min. The specificity of the method was tested by analyzing horse and
82 donkey plasma samples before the administration of IOX. No interfering peaks were observed at the
83 elution times of IOX or IS isomers. IOX limit of detection (LOD) and limit of quatification (LOQ)
84 were found to be 0.01 and 0.1 µg/ml, respectively. Calibration graphs for IOX (n = 9), constructed
85 over the concentration range of 0.5-500 µg/ml, showed an average correlation coefficient (R^2) of
86 0.99. Plasma recovery for both substances was 90±3%. The intra- and inter-assay coefficients of
87 variation (CVs) were <10% for each compounds.

88 *Preparation of plasma samples.* Fifty microliters of plasma were added to a 50 µl water solution of

IS (50 µg/ml) and vigorously vortexed (30''). The plasma sample was deproteinized by adding 100 µl of dichloromethane (CH_2Cl_2), extracted with double-distilled water (150 µl), vigorously vortex (30'') and centrifuged at 3500 rpm for 10 minutes. Twenty microliters of the supernatant were centrifuged at 3000 rpm for 10 minutes and then injected into the HPLC system.

Pharmacokinetic and statistical analysis

Pharmacokinetic analyses were performed by WinNonlin Version 5.1^n. The data were analysed by nonlinear least squares regression analysis with equal weighting of the data. The data points were weighted by the inverse of the square-fitted value. The best fit was obtained by minimizing the weighted least-square criteria, and the number of exponents (1, 2 or 3) needed for each data set was determined by application of the Akaike's information criterion.[29] A biexponential equation was selected on the basis of this criterion for intravenous administration of IOX: $Ct=C1 \times e^{-\lambda 1t} + C2 \times e^{-\lambda 2t}$, where Ct is the plasma concentration at any time t, described the data for each animal. Pharmacokinetic variables were then calculated using the intercepts (C1 and C2) and absolute values of the slopes (λ1 and λ2) of the best fit equation for each horse. The area under the plasma concentration versus time curve (AUC) was calculated from the intercepts and slopes of the biexponential equations for each individual animal according to $AUC = C1/\lambda 1 + C2/\lambda 2$. The total plasma clearance (Clt) was calculated from Clt = dose/AUC. Derived parameters (volume of distribution, MRT) were calculated according to standard procedures for compartmental analysis.[30] The normalized nine-point clearance value was considered a reference for the evaluation of simplified methods. Correlation analysis between the GFR values obtained by the nine-point clearance method and the GFR values determined by the application of simplified sample combinations were performed using Pearson test, linear regression analysis and One-way ANOVA test.

For simplified methods GFR was calculated by using one-compartment model. However, this formula overestimates clearance because it does not consider plasma concentrations during the

distribution phase. Therefore, the GFR was corrected by the following empirically determined formula[31]: GFR = 0.991 x Cl – 0.00122 x $(Cl)^2$. Among the possible different models, six simplified sample combinations (Model A, B, C, D, E and F) were chosen. Each model showed a different sample combination: Model A (5, 30, 60, 90 and 240 minutes), Model B (5, 30, 60, 90 and 120 minutes), Model C (5, 30, 60 and 90 minutes), Model D (5, 30, 90 and 120 minutes), Model E (5, 30 and 90 minutes) and Model F (5, 30 and 120 minutes).

Plasma clearance of IOX was determinated by dividing dose administered by AUC and indexed to body weight (BW) (ml/min/kg). Results are presented as mean±SD. Comparison of values between horses and donkeys was performed using a t-test. A p value lower than 0.05 was considered significant.

RESULTS

The plasma concentration *vs* time profiles for IOX obtained from nine-point clearance method (5, 15, 30, 45, 60, 90, 120, 240 and 360 minutes) in all analyzed horses and donkeys are reported in Figures 1 and 2, respectively. Plasma clearance of IOX in horses was 597.0±298.5 ml/min and, when adjusted for body weight of each animal, the GFR was calculated to be 1.19±0.21 ml/min kg (Table 1). Plasma clearance of IOX in donkeys was 630.8±35.8 ml/min and, when adjusted for body weight of each animal, the GFR was calculated to be 1.80±0.10 ml/min kg (Table 2). The plasma concentration of IOX was below the LOQ of the HPLC method 12 h after its intravenous administration for both horses and donkeys. The mean pharmacokinetic parameters values are shown in Tables 3 and 4. GFR was significantly lower ($p < 0.05$) in horses in comparison with donkeys.

DISCUSSION

Measurement of glomerular filtration rate (GFR) in horses is not frequently done for research or clinical cases because of the difficulties involved in performing renal function tests. Conventional renal clearance techniques for the measurement of glomerular filtration rate (GFR) pose potentially daunting problems in horses. This paper reported the determination of GFR in adult horses and donkeys by single intravenous administration of IOX. No adverse clinical signs related to IOX injection were seen throughout the experiment period and the test has been easily performed in both species. In the horse, GFR has been evaluated by different methods. GFR evaluated by single injection serum clearance of inulin resulted 3,21+0,36 ml/kg/min in ponies[31] and 2,30+0,34 in horse and pony foals,[10] while GFR obtained by use of plasma clearance of technetium 99m pentetate was 1,83+0,20 ml/Kg/min. GFR obtained in this study in adult horses was 1.10±0.23 ml/Kg/min, while in donkeys our results showed a IOX clearance of 1.76±0.30 ml/Kg/min. Our results on IOX clearance seemed to be in line to Matthews' results[4] using radio pharmaceuticals, both for horses and donkey. The use of radio pharmaceuticals compares well with standard methods for GFR measurement in people[33] and animals,[4,12] but these substances are potentially toxic, while IOX is safe. IOX plasma clearance has been evaluated in equine specie. In particular, the evaluation of IOX clearance used to estimate GFR has been carried out adult horses[25] and in equine foals.[24] The mean serum IOX clearance was 2,38 ml/Kg/min in adult horses and 2,15 ml/Kg/min in foals. Our results on IOX clearance rate in adult horses showed lower values if compared to values reported by others.[4,24-25] These variances might be related to the different dosage used in this study (75.5 mg/Kg) in comparison to previous works performed in the adult horses[25] and foals[24] (150 mg/Kg). The differences might also be due to the use of a biexponential equation to fit the serum IOX concentration versus time, while triexponential equation has been used in all the previous studies.[24-25] The results on GFR in donkeys cannot be compared to other previous works because to the authors' knowledge there is no literature concerning GFR in donkeys. In our study, the plasma clearance rate of IOX resulted significantly greater in donkeys then in horses. Moreover, our results

on GFR in donkeys showed higher values than in horses reported by others.[24-25] These differences might be due to the weight and body surface.[1]

In conclusion, IOX clearance could be a good method for the evaluation of GFR in clinical cases because the test is easy to perform and it determines GFR rapidly with minimal blood sampling and the urine collection is not necessary. Moreover, IOX plasma clearance shows greater precision than conventional inulin clearance, probably because it avoids the errors associated with complete urine collection.[4]

Footnotes

[a]kinetic modified Jaffè method, cod. ASR01150, Assel Srl, Rome, Italy; [b]kinetic enzymatic method, cod. ASR01143, Assel Srl, Rome, Italy; [c]Reagent Strip Analysis, …; [d]14G, Terumo, Japan; [e]Omnipaque 350, Nycomed Imaging AS, Oslo, Norway; [f]cod. 22304, FL Medical, Padua, Italy; [g]Nycomed Amersham Sorin[g] (Milan, Italy); [h]Goettingen, Germany; [i]LABSCAN, Hasselt, Belgium; [l]Waters, Milford, MA, USA; [m]MS OFFICE, 2008; [n]Pharsight, Mountain View, CA.

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Figure legend

Figure 1. Plasma eso-iohexol concentration vs. time profile from 8 horses after a single administration of IOX (75.5 mg/kg); data are expressed as mean±standard deviation bars.

Figure 2. Plasma eso-iohexol concentration vs. time profile from 8 donkeys after a single administration of IOX (75.5 mg/kg); data are expressed as mean±standard deviation bars.

261 Table 1. GFR values of horses expressed as ml/min*kg.

Horse	GFR (ml/min*kg)
1	0.87
2	0.80
3	0.90
4	1.05
5	1.45
6	1.17
7	1.22
8	1.30
Median	1.11
Mean	1.10
Min	0.80
Max	1.45

262 Table 2. GFR values of donkeys expressed as ml/min*kg.

Donkey	GFR (ml/min*kg)
1	1.98
2	1.32
3	1.55
4	1.78
5	2.06
6	1.46
7	2.19
8	1.71
Median	1.75
Mean	1.76
Min	1.32
Max	2.20

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265 Table 3. Mean pharmacokinetic parameters values of horses; AUC, area under the curve; Cl,
266 clearance; Vd, volume of distribution; MRT, mean residence time.

Horse	AUC (min*mg/ml)	Cl (ml/min)	Vd (ml)	MRT (min)
1	21800.0	435.8	70080.0	143.4
2	24916.7	400.0	52000.0	124.8
3	22783.3	450.0	50600.0	102
4	19000.0	522.5	88360.0	94.2
5	13616.7	725.0	89610.0	114.0
6	16950.0	583.3	97780.0	111.0
7	18666.7	608.3	88500.0	105.0
8	20500.0	648.3	90100.0	114.0
Median	19750.0	553.0	88430.0	112.5
Mean	19783.3	546.7	78380.0	113.5
Min	13616.7	400.0	50600.0	94.2
Max	24916.7	725.0	97780.0	143.4

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268 Table 4. Mean pharmacokinetic parameters values of donkeys; AUC, area under the curve; Cl,
269 clearance; Vd, volume of distribution; MRT, mean residence time.

Donkey	AUC (min*mg/ml)	Cl (ml/min)	Vd (ml)	MRT (min)
1	8570.0	754.0	94150.0	93.6
2	13006.7	500.7	58270.0	97.2
3	10950.0	589.0	77740.0	97.2
4	8016.7	677.8	86760.0	106.2
5	8333.3	783.3	97120.0	91.2
6	10835.0	553.3	75600.0	81.0
7	7400.0	830.8	132000.0	132.0
8	9270.0	648.3	95200.0	108.0
Median	8920.0	663.2	90460.0	97.2
Mean	9548.3	667.2	89610.0	100.8
Min	7400.0	500.7	58270.0	81.0
Max	13006.7	830.8	132000.0	132.0

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