New high-performance liquid chromatographic method for therapeutic drug monitoring of isepamicin

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HPLC method with derivatisation for measuring isepamicin concentration in serum has been developed. The assay utilised extraction of isepamicin and tobramicin (IS) from serum on Amberlite XAD-2 columns. Derivatisation was performed with 1-fluor-2,4-dinitrobenzene for 1 hour in water bath at 80 °C. Chromatography was carried out using microbore column (1 × 150 mm) with reverse octadecyl phase and acetonitril/methanol/water/triethylamine 20:80:140:0,05 v/v/v/v, pH 3,8 as mobile phase. Separation was monitored at 365 nm. Linearity was 0–100 mg/ml, recovery 96–102% and coefficient of variations about 5% in tested concentrations (10, 25 and 100 mg/ml).

Trough and peak serum levels of isepamicin were measured during the treatment of 17 paediatric patients (8 were under 6 and 9 over 6 years). Mean age was 8,4±5 years and weight 26,7±13.4 kg. The dose of isepamicin 13,3±2,9 mg/kg was given in 30 minutes infusion once daily. Trough level was found 1,05±1,51 mg/l and peak level 35,4±19,5 mg/l, respectively.

Pharmacokinetic data were obtained by the aid of pharmacokinetic program MW-PHARM 3.30. Gentamicin population pharmacokinetic model for different age groups was used. In the group of children under 6 year the pharmacokinetic data were CL 2,41±1,71 ml/min/kg, Vd 0,49±0,12 l/kg, $t_{½}$ 3,03±2,12 hours, C_{max} 28,77±19,46 mg/l, C_{min} 0,58±0,75mg/l and t_{max} 0,51±0,24 hours. While in the group of children over 6 year were CL 1,50±1,45 ml/min/kg, Vd 0,32±0,08 l/kg, $t_{½}$ 3,83±1,97 hours, C_{max} 41,23±23,81 mg/l, C_{min} 1,47±1,92 mg/l and t_{max} 0,40±0,17 hours.

Implementation of HPLC method and pharmacokinetic modelling may reduce risk of isepamicin toxicity.

Key words: isepamicin, HPLC, TDM, pharmacokinetics, children.

NOVÁ METODA KAPALINOVÉ CHROMATOGRAFIE PRO TERAPEUTICKÉ MONITOROVÁNÍ ISEPAMICINU

Na stanovení koncentrace isepamicinu v séru byla zavedena HPLC metoda s derivatizací. Isepamicin a tobramicin (vnitřní standard) se extrahovaly ze séra na kolonkách plněných Amberlitem XAD-2. Derivatizace se prováděla 1-fluor-2,4-dinitrobenzenem 1 hod na vodní lázni při teplotě 80 °C. Na chromatografickou analýzu se použila microbore kolona 1x150 mm s reversní octadecylovou fází, mobilní fáze byla složena následovně: acetonitril/methanol/voda/triethylamin 20:80:140:0,05 v/v/v/v, pH 3,8. Detekce se prováděla při 365 nm. Linearita metody byla testována v rozsahu 0–100 mg/ml, recovery bylo 96–102% a variační koeficient okolo 5% v koncentracích 10, 25 a 100 mg/l.

U 17 pediatrických pacientů (osm bylo mladších než šest let a devět starších) byla stanovována minimální a maximální koncentrace isepamicinu. Průměrný věk dětí byl 8,4±5 let a hmotnost 26,7±13,4 kg. Dávka 13,3±2,9 mg/kg isepamicinu se podávala ve 30 minutové infuzi jednou denně. Průměrné údolní koncentrace isepamicinu byly 1,05±1,5 mg/l a v píku 35,4±19,5 mg/l.

Farmakokinetické parametry se získaly pomocí farmakokinetického programu MW- PHARM 3.30. Jako model se použila populační farmakokinetika gentamicinu pro různé věkové skupiny. U dětí pod šest let jsme zjistili následující farmakokinetické parametry: CL 2,41±1,71 ml/min/kg, Vd 0,49±0,12 l/kg, $t_{½}$ 3,03±2,12 hod., C_{max} 28,77±19,46 mg/l, C_{min} 0,58±0,75mg/l a t_{max} 0,51±0,24 hodin. U dětí nad 6 let: CL 1,50±1,45 ml/min/ kg, Vd 0,32±0,087 l/kg, $t_{½}$ 3,83±1,97 hod.,C_{max} 41,23±23,81 mg/l, C_{min} 1,47±1,92 mg/l a t_{max} 0,40±0,17 hod.

Zavedení HPLC metody a farmakokinetické modelování může snížit riziko toxicity isepamicinu.

Klíčová slova: isepamicin, HPLC, TDM, farmakokinetika, děti.

Isepamicin is a new aminoglycoside antibiotic with broad spectrum of activity against both gram-positive and gram-negative bacteria. Its structure is near to gentamicin and so some pharmacokinetic parameters. Its toxicity is lower while resistance to degrading enzymes is higher which is caused by structural changes in the molecule. Isepamicin is not modified by the 2'-aminoglycoside adenyltransferase or aminoglycoside acetyltransferases that inactivate gentamicin, and it is also less affected by the 6'-aminoglycoside acetyltransferase inactivating amikacin. As a result, the frequency of strains resistant to isepamicin is lower than it is in the case of gentamicin or amikacin[1, 2].

Minimal inhibition concentration (MIC) of isepamicin is the same or slight lower to compare with other aminoglycoside antibiotics and the efficacy of the treatment is comparable by once or twice daily dosing regimen[3]. Monitoring trough and

peak isepamicin concentration 30 minutes after iv application is recommended as it is in patients with other aminoglycoside antibiotics. Trough and peak safety levels should be below 10 and 80 mg/l, respectively. Persistently elevated concentrations may cause renal impairment and central nervous system toxicity and thus monitoring of vestibular and auditory functions is recommended[2, 4].

Concentrations of gentamicin and amikacin are usually measured by immunoanalytical methods, but immunoanalytical method for isepamicin has not been available in our country. Therefore isepamicin level can be estimated either by high-performance liquid chromatography (HPLC) or agar diffusion bioassay using a *Staphylococcus epidermis* strain[2]. Original HPLC method for isepamicin from Schering-Plough laboratories[5, 6] used an automated column switching HPLC technique and post column derivatisation was performed by continual addition of o-phthaladehyde. Dionissoti[7] used as derivatisating agent dinitrofluorbenze and measured isepamicin in whole serum without foregoing extraction. Maloney extracted isepamicin on cyano columns before derivatisation, but this cleaning up step revealed low recoveries[8].

The aim of this study was: 1. To develop HPLC method for analysis of isepamicin for therapeutic drug monitoring (TDM); 2. To find pharmacokinetic model for prediction of isepamicin serum level.

Material and method

Methanol and acetonitrile, both HPLC grade were obtained from Merck, water HPLC grade and 1-fluor-2, 4-dinitrobenzene was purchased from Sigma-Aldrich and Fluka Chemie, acetic acid analytical grade and triethylamine were from Lachema. Isepamicin in concentration 250 mg/ml ISEPACIN® Schering-Plough was used for preparing stock solution. Tobramicin used as internal standard was obtained from Biogal as BRULAMICIN®.

The stock solutions of isepamicin and tobramicin were prepared in water at concentrations 1mg /ml and were kept at 8 °C. These solutions are stable at least four months storing in refrigerator. Standard samples of isepamicin were prepared in serum of untreated volunteer in concentrations 2,5, 5, 10, 25, 50, 75 and 100 mg/l and were processed the same way as patients´ samples.

Tab. No. 1. Chacterictics of cohort (age, body weight, dose/kg, creatinine, isepamicin concetration – trough and peak, further antibiotics)

No.	age (years)	weight (kg)	dose/kg (mg/kg)	day No.	creatinine (µmol/l)	levels of isepamicin (mg/l)		other ATB
						trough	peak	
1.	6	25	15,0	7 12 17	48	1,6 2,0 0,8	 25,4	CFZ
2.	11	35	14,3 7,1	6 8	203 203	 4,5	 39,7	PIP + TZB
3.	14	50	10,0	8 13 15	 58 54	0,1 0,9 0,9	13,5+ 34,5 24,6	CFZ
4.	5	18	11,1	3 5	47	0,2 0,2	18,5 17,0	CFZ
5.	3	10	15,0	5 8	56	0,2 0,2	14,5+ 24,6	CFZ FLC
6.	14	30	16,6	6	42	4,4		MRP
7.	5	15	13,3	8 12	56 45	0,1 0,6	9,5+ 19,6	CFZ
8.	11	45	16,6	3	70	2,9	48,3	
9.	4	13	19,2	5 6	63	1,1 1,1	45,6 22,1+	CFZ
10.	8	23	15,2	8	43	0,3	33,0	MRP
11.	14	45	11,1 10,0	6 10	111 69	0,2 0,1	21,6+ 22,1+	PIP + TZB
12.	2	9	13,9	5		1,2	34,5	
13.	7	21	12,0	4 7 9	39 50 47	0,2 1,1 1,2	14,2+ 19,0+ 11,6+	SLT, MRP
14.	13	35	14,3	4	56	0,4	30,8	CFZ, FLC
15.	2	6	15,0	3	76	1,8	26,1	CFZ
16.	6	20	12,4	6		0,3	22,4	
17.	14	50	10,0	6 8	64	0,1 0,1	28,0 22,0	PIP + TZB

CFZ ceftazidim, FLC fluconazolum, MRP meropenem, PIP piperacilinum, SLT sultamicilinum, TZB tazobactam;
+ peak blood level taken after 60 min

Analysis of Isepamicin

HPLC method with derivatisation for measuring isepamicin concentration in serum has been developed. The assay utilised extraction of isepamicin and tobramicin (internal standard, IS) from serum on Amberlite® XAD-2 columns (Applied Separations), which were previously conditioned by two volumes of methanol, water and 0,02 mol/l acetic acid. To vials with tobramicin as internal standard (concentration 25mg/l) 0,5 ml of serum and the same volume of 0,02 mol/l acetic acid were added. Samples were vortexed, centifugated and applied on the top of columns. The occurring substances were washed out by 750 µl 0,02 mol/l acetic acid and isepamicin and tobramicin were eluted with 250 µl of acetonitril. Derivatisation was performed with 1-fluor-2,4-dinitrobenzene in water bath at 80 °C for one hour. 20 µl of reaction mixture was injected for analysis.

Chromatographic conditions

Chromatographic equipment (TSP) consisting of isocratic pump SP 1500, UV-VIS detector UV1000 and autosampler AS 1000 was used. Chromatography was carried out using microbore column and precolumn (1 × 150 mm and 1 × 30 mm, respectively, Tessek) with reverse octadecyl phase and acetonitril/methanol/water/triethylamine 200: 80:140:0,05 v/v/v/v, was used as a mobile phase, pH was adjusted to 3,8 with concentrated acetic acid. Rate of mobile phase was 0,13 ml/min. and separation was monitored at 365 nm.

Cohort

Isepamicin levels were repeatedly measured during the treatment in a group of 17 children with severe bacterial infections. Children were treated by isepamicin alone in 30 minutes infusion once daily or in combinations with other antibiotics. Dose of isepamicin per day was 125–750 mg according to the age, body weight and severity of infection and each application was consulted with antibiotic centre. Therapeutic effect was in all patients satisfactory.

Tab. No. 2. Cohort (mean ± SD divided according to the age of paediatric patients)

	all children	under 6 years	over 6 years
number	17	8	9
age	8,4 ± 5 (2–14)	4,1 ± 1,6 (2–6)	11,8 ± 2,7 (7–14)
weight	26,7 ± 13,4 (6–50)	14,6 ± 6,4 (6–25)	37,0 ± 11,2 (20–50)
creatinine	66,1 ± 36,8 (39–203)	52,7 ± 10,6 (39–76)	75,9 ± 46,0 (43–203)
dose/kg	13,3 ± 2,9 (7,1–19,2)	14,3 ± 2,4 (11,1–19,2)	12,5 ± 3,1 (7,1–15,2)

Tab. No. 3. Recovery and coefficient of variations of isepamicin (three levels were used 10, 25 and 50 mg/l)

concentration tested (mg/l)	10	25	50
concentration measured (mg/l)	10,2	24,8	48,4
n	6	6	6
recovery (%)	102,0	99,1	96,8
CV (%)	5,4	7,0	5,7

Information about the cohort is given in Tab. No. 1. Mean values are summarised firstly from all children and than divided into two groups according to their age in Table No. 2.

Blood samples were taken 30 minutes before administration of the next dose of isepamicin and 30 or 60 minutes after application. All samples were analysed in duplicates by HPLC method.

Pharmacokinetic analysis

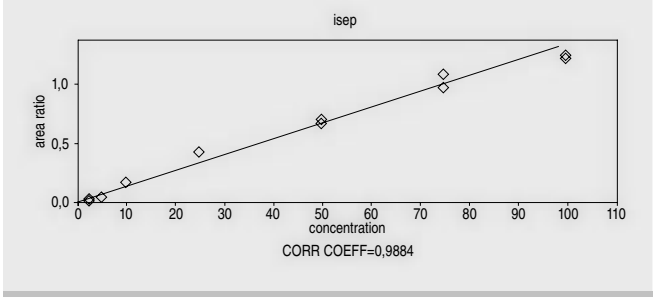
Pharmacokinetic parameters were calculated with the aid of MW-PHARM 3.30 software, which is based on Bayesian population pharmacokinetics[9]. Because of isepamicin is new antibiotic and no available data of population pharmacokinetic exist, we used population pharmacokinetics of gentamicin as a model. According to this program the children were divided into three groups on the bases of their age: 1–5, 5–10 and 10–16 years. From the trough and peak levels of isepamicin the individual pharmacokinetic parameters were calculated for each child.

Results

Linearity of the method is shown on Fig. No. 1. Recovery and coefficient of variations respectively in tested concentrations (10, 25 and 100 mg/l) are in Tab. No. 3. Limit of quantification defined as the lowest concentration that yields coefficient of variations less than 20 % and accuracy between 80–120 % was determined as 0,2 mg/l. Examples of chromatograms (serum of untreated volunteer and low and high levels of isepamicin analysed as trough and peak ones, respectively) are given in Fig. No. 2.

Pharmacokinetic data calculated on the bases of pharmacokinetic software MW-PHARM 3.30 for all children as well as divided into two groups according to their age (under six years and over six years) are summarised in the Tab. No. 4. Younger children have higher values of clearance and volume of distribution, lower elimination half-life and trough and peak concentrations, respectively. Curves of isepamicin levels during the treatment are shown in two children on the Fig. No. 3. Concentration of isepamicin after administration depended on the time period between application and taking blood samples. In samples taken one hour after infusion were concentrations of isepamicin roughly half of those in 30 minutes. The course of isepamicin level is also in close relation with serum creatinine as it is shown in the case of No. 2, where dose of isepamicin had to be reduced owing to elevated creatinine value.

Fig. No. 1. Reference curve of isepamicin



Tab. No 4. Pharmacokinetics parameters calculated by using pharmacokinetic model of gentamicin (mean±SD)

	CL (ml/min/kg)	Vd (l/kg)	$t_{½}$ (hod)	C_{max} (mg/l)	C_{min} (mg/l)	t_{max} hod
all patients	1,93 ± 1,60	0,40 ± 0,14	3,45 ± 2,02	35,36 ± 19,46	1,05 ± 1,51	0,45 ± 0,20
1–6 years	2,41 ± 1,71	0,49 ± 0,12	3,03 ± 2,12	28,77 ± 19,46	0,58 ± 0,75	0,51 ± 0,24
> 6 years	1,50 ± 1,42	0,32 ± 0,08	3,83 ± 1,97	41,23 ± 23,81	1,47 ± 1,92	0,40 ± 0,17

Fig. No. 2. The examples of chromatograms a) serum of non-treated volunteer without any drug, b) serum with low (trough) isepamicin level, c) serum with high (peak) isepamicin level; is – internal standard- tobramicin, isep–isepamicin

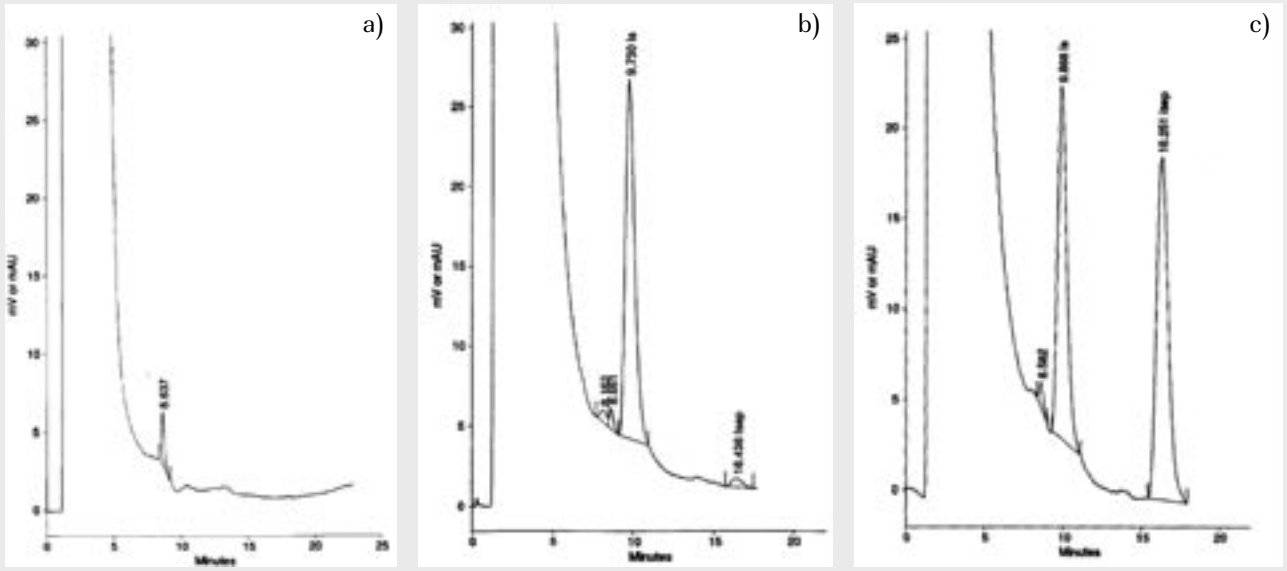
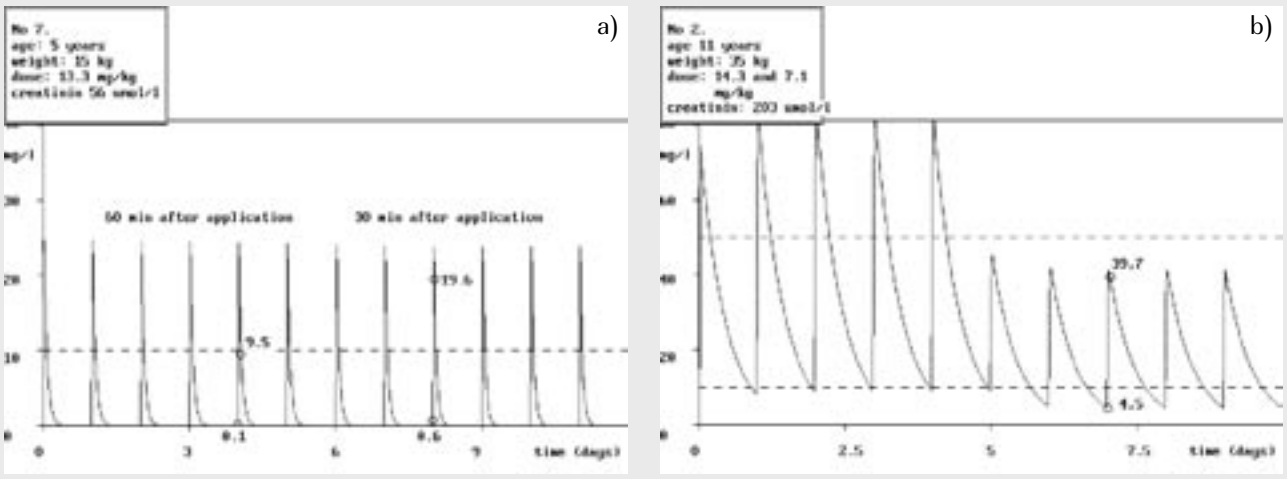


Fig. No. 3. Pharmacokinetic models of isepamicin level (x time in day, y concentration mg/l) a) Child No. 7: Concentration was measured 60 and 30 minutes after application b) Child No.2: Isepamicin concentration was influenced by elevated serum creatinine. Reduction of isepamicin dose is shown as a result of pharmacokinetic analysis. New isepamicin concentration agrees with predicted value



Discussion

Several methods for the determination of isepamicin in serum have been described including immunoanalytical method, HPLC and agar diffusion bioassay. The most convenient is fluorescence polarisation immunoassay as it is in the case of other aminoglycoside antibiotics[2]. Because we were not able to purchase immunoanalytical set for isepamicin analysis, HPLC method for determination serum levels for TDM had to be developed.

Isepamicin itself has neither absorbance nor fluorescence in the appropriate spectral range and therefore must be derivatised by suitable derivatisation agent, 1-fluor-2,4-dinitrobenzene[7], or o-phthaladehyde[5, 6, 8]. Original HPLC method from Schering – Plough laboratories[5, 6] based on automated column switching technique, postcolumn derivatisation and fluorescence detection was not convenient according to our laboratory equipment. Dionissoti[7] used precolumn derivatisation and measured isepamicin by the aid of UV VIS detector

in whole serum without foregoing extraction. Maloney extracted isepamicin on CN cartridges but this cleaning up step revealed low recoveries, only about 5 %[8]. We modified method of Dionissoti using extraction step on Amberlite® XAD-2 columns before derivatisation. Linearity of the method, limit of quantification, recovery and coefficient of variations fulfilled demands on analytical method.

The next step was to find convenient pharmacokinetic model for predicting the course of the isepamicin serum concentration. Because of TDM of aminoglycoside antibiotics (gentamicin and amikacin mainly) has been provided for many years by the aid of pharmacokinetic program MW-PHARM[9], we tried to use this software also in the case of isepamicin. Population pharmacokinetic model of gentamicin according to the age of treated children solved this problem better to compare with those of amikacin or netilmicin. Higher clearance, volume of distribution and lower half-life of elimination and both maximal and minimal concentrations in younger children are in agreement with knowledge about increased metabolism and excretion in this age group[10, 11]. Also single pharmacokinetic parameters are comparable with those published by other authors. Tod et al. presented for the same age groups (children under and over six years) clearance 2,64 and 2,60 ml/min/kg, volume of distribution 0,43 and 0,41 l/kg and peak concentration 37 and 39 mg/l, respectively[2]. Comparing this data with our results, where clearance was 2,41 and 1,50 ml/min/kg, volume of distribution 0,49 and 0,32 l/kg and peak concentration 28,8 and 41,2 mg/l respectively, it was shown that namely in the group of younger children are the data similar. Pharmacokinetic parameters for older children are more close to data of younger adult volunteers (between 21–30 years) with clearance 1,67 ml/min/kg, volume of distribution 0,33 l/kg and peak concentration 66,3 mg/l, but the dose per kg was in volunteers higher 15 mg against 12,5 mg[12]. Half-life of elimination was in both our groups higher (3,0 and 3,8 hours, respectively) to compare with half-life about two hour in studies of previous authors[2, 12]. Only Radwanski found elimination half-life 3,6 hour after single iv administration of isepamicin to adult volunteers[1].

Although the recommended interval for taking blood samples in intravenous application is 30 minutes[2, 4], in some children namely in the beginning of the study was the blood taken later (one hour), which was reflected in lower isepamicin levels. By using pharmacokinetic modelling these discrepancies in timing are eliminated[9]. The course of isepamicin level during the treatment is in close relation with creatinine value as it is in the case No. 2 where necessity of reduction of isepamicin dose is shown. But this problem occurs more often in adult patients, where renal failure with high serum creatinine is more common and namely patients with dialysis have to be treated with aminoglycoside antibiotics by special dosing regimen[2, 4].

Developing HPLC method for isepamicin, monitoring trough and peak levels and their prediction with the aid of pharmacokinetic modelling may reduce risk of neural and renal impairment which can be induced by inappropriate high serum concentrations.

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