Original Paper

Pharmacokinetic profile evaluation of di-μ-hydroxo-bis(quercetinatooxovanadium(IV)) complex

B.S. VELESCU[1], VALENTINA UIVAROSI[1], ANCA BUZESCU[1], I. SARBU[1], ECATERINA IONESCU[2], VALENTINA ANUTA[1]

1 "Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy
2 "Carol Davila" University of Medicine and Pharmacy, Faculty of Dental Medicine

ABSTRACT: The present study was designed to evaluate the pharmacokinetic (PK) properties of di-μ-hydroxo-bis(quercetinatooxovanadium(IV) (HOBQOV) complex, with hypoglycemic and hypolipidemic properties, in rats after a single i.p. dose of 100 mg/kg b.w. The HOBQOV complex pharmacokinetics is described by a bicompartmental model and the plasma concentration decrease is described by two phases that might be correlated to the distribution and elimination processes. The obtained results suggest a PK profile of the complex suitable for a profound distribution in the animal organism with possibility of interaction with the cytosolic enzymes.

***KEYWORDS**: HOBQOV, HPLC, pharmacokinetics, bicompartmental model*

Introduction

Diabetes mellitus is a metabolic disorder with increasing prevalence estimated by World Health Organization to affect by 2030 over 350 million patients worldwide [1]. The pharmacological agents used for diabetes treatment present multiple adverse reactions [2] with different intensity based on the patient's posology and individual reactivity. Alternative approaches, based on the design of transitional metal complex combination (Zn, V, Cu, Cr), for diabetes treatment have been investigated [3-4]. The benefic effects of vanadium in streptozotocin induced diabetes models [5] and the inclusion of bis(ethylmaltolato)oxovanadium(IV), in clinical trials [6] supported further research in the development of pharmacological active vanadium complexes. Vanadium compounds pharmacodinamy is correlated with the interaction of vanadium species with different cytosolic enzymes [7]. In this way an adequate pharmacokinetic with a high intercellular distribution profile, able to facilitate the cellular intake of the complex, is required for exerting the biologic activity. A series of $[VO(L)_2]$ complexes with flavonoids as ligands were synthesized and characterized [8-9]. The complex di-μ-hydroxo-bis(quercetinato oxovanadium(IV)) (HOBQOV) (Fig.1) improved the impaired blood glucose and lipidic profiles [10], The present study was designed to evaluate the pharmacokinetic (PK) properties of a HOBQOV complex in rats after a single i.p. dose of 100 mg/kg b.w. The complex kinetics is described by a bicompartmental model. The obtained results suggest a PK profile of the complex suitable for a profound distribution in the animal organism with possibility of interaction with the cytosolic enzymes.

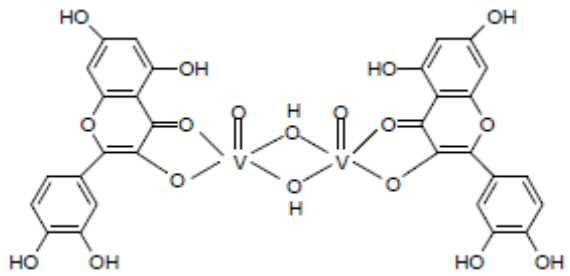


Fig.1. Structure of di-μ-hydroxo-bis(quercetinatooxovanadium(IV)

Materials and Methods

Materials

HOBQOV complex was synthesized and physico-chemical characterized according to a method described in a previous paper [8].The solvents used (acetonitrile, methanol, dimethylsulfoxide, diethyl-ether, hexane) of HPLC grade were purchased from Sigma-Aldrich. Analytical grade trifluoroacetic acid was purchased from Scharlau.

Experimental animals

Female Wistar rats weighing (142 ± 13 g) from the Cantacuzino Institute, Bucharest were used. The rats were housed in plastic cages in an air-conditioned animal room and fed on granulated food with free access to water.

The temperature and relative humidity were continuously monitored using a thermohygrometer. The temperature was

between 20ºC and 22ºC and the relative humidity was generally maintained at 35-45%.

All procedures were carried out in accordance with the Directive 2010/63/EU, governing animal research in Europe (revising Directive 86/609/EEC), on the protection of animals used for experimental and other scientific purposes.

Pharmacokinetics - Study design

28 female Wistar rats were randomly distributed in 5 groups of 5 animals and 3 animals were used as blank.

The animals were administered a dose of 100 mg/b.w. of HOBQOV complex, by intraperitoneal injection. The administered dose represented 1/20 from maxim administered dose that proved no lethal effect. Following the administration, the animal groups were sacrificed at 30 min, 1h, 2h, 6h and 24h respectively. The blood was collected on Na_2EDTA and was centrifuged; plasma samples were stored at -20ºC for HPLC analyses.

Pharmacokinetic analysis was performed with Kinetica 2000 software.

HPLC assay - HPLC conditions

The analyses were carried out using a Waters Liquid Cromatograph (600E Multisolvent Delivery System, 717 Autosampler, 486 Tunable Absorbance Detector, Waters, Milford, MA, USA. Empower Software package (Waters, Milford, MA, USA) was used to control instrument, data acquisition and data analysis. The UV detector was set at 281 nm.

The chromatographic separation was achieved on a Kromasil 100-5C18, 5-μm 150 x 4 mm column (Akzo Nobel) using as mobile phase an isocratic mixture of 0.1% trifluoroacetic acid:acetonitrile (30:70 v/v), delivered at 1.0 ml/min flow rate, al 25ºC. 100 μl of each sample was injected into the chromatographic column, for a run time of 12 min.

Plasma standards preparation

Standard plasma samples: stock solution containing 250 μg/ml of HOBQOV were made by dissolving 10.0 mg of HOBQOV in dimethylsulfoxide in a 25 ml volumetric flask. Further dilutions were made with blank plasma, in order to obtain standard plasma samples in the range 0.01-50 μg/ml. Separate solutions were prepared for the calibration curve samples and quality control.

Sample preparation

To 0.5 mL of plasma sample, 250 μl of 0.2M phosphate buffer pH 5.4 were added. The samples were vortex-mixed for homogenization, and 3 ml diethylether:hexane mixture (7:3 v/v) was added. The samples were vortexed horizontally for 20 minutes at 120 rpm, and 2.5 ml of the organic layer was transferred into a conical tube, and evaporated to dryness under nitrogen stream, at 45ºC. The dry residue was reconstituted in 300 μl of mobile phase. An aliquot of 100 μl from the resulting solution was injected into the chromatographic system.

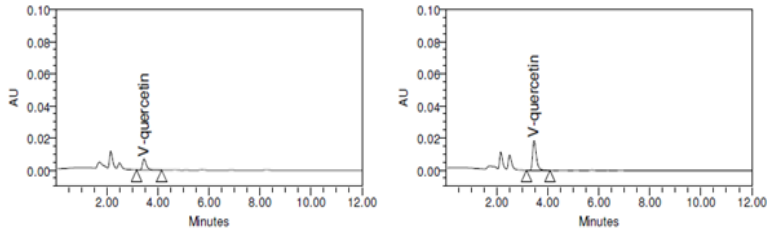
Pharmacokinetic analysis

PK analysis was carried out by using non-compartmental (NC) and compartmental (C) methods.

NC analysis was used for the estimation of half time ($t_{½}$), mean residence time (MRT), total clearance (Cl_T), volume of distribution (V_d), area under the time-concentration curve (AUC_{0-24}).

C analysis was used for the estimation of the proper compartmental model followed by the complex and macro and micro constants associated to the identified model.

The compartmental model was estimated by using the Marquardt curve fitting algorithm. The Akaike information criterion and Schwarz criterion [11] were used for identifying the optimum model describing the complex kinetics.



	SampleName	Peak Name	Area
1	Vq 5ppm	V-quercetin	71429
2	Vq 10ppm	V-quercetin	182198

Fig.2. Representative chromatogram for the plasma analyses of HOBQOV complex

DOI: 10.12865/CHSJ.40.04.06

Results

Analytical method validation

The HPLC method for the quantification of HOBQOV complex from plasma samples was validated in accordance with international regulations [12-13]. A representative chromatogram of for the plasma analysis of HOBQOV complex is presented in Fig.2.

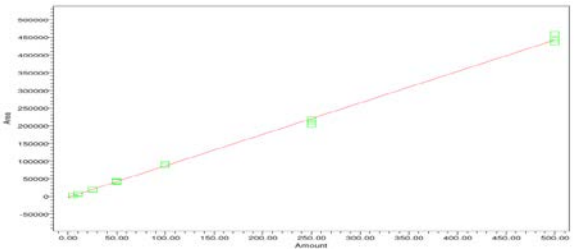


Fig.3. Calibration plot of the method

The calibration curve (Fig.3) for HOBQOV complex in plasma was generated by using seven concentration levels, in the range 5 – 500 ng/ml. Three replicates for each concentration were used. The calibration curve was linear (r>0,9999) with a lower limit of quantification (LLOQ) of 5ng/ml. For LLOQ concentration, the precision (characterized by the relative standard deviation) was 3.77%, and accuracy (defined as the deviation between the true and the measured value expressed in percent) was -1.49%.The intra-assay precision and accuracy was estimated by analyzing the quality control samples (low, medium and high concentration) five times in the same analytical run. Both intra-assay accuracy and precision were within the accepted limits (Table 1). The precision was better than 3% and the bias did not exceed 5% at all concentration levels tested.

The recovery from plasma was influenced by the protein plasma binding of HOBQOV (82 % mean recovery); nevertheless the value of the recovery is appropriate for the determination of plasma level concentrations of the analyte.

Table 1. Intra-assay accuracy and precision of the method

Concentration (µg/mL)			
Added	**Measured**	**Accuracy (%)**	**RSD (%)**
300	298.90	-0.36	4.4
60	61.56	2.60	2.6
15	15.28	1.91	3.3

Samples analysis

The HPLC method was applied in an experimental PK study for the estimation of HOBQOV complex PK parameters, following a single dose administration.

Representative chromatograms of HOBQOV complex plasma samples are presented in Fig. 4.

The mean plasma concentrations of HOBQOV complex according to the sampling moments, are represented in Fig. 5.

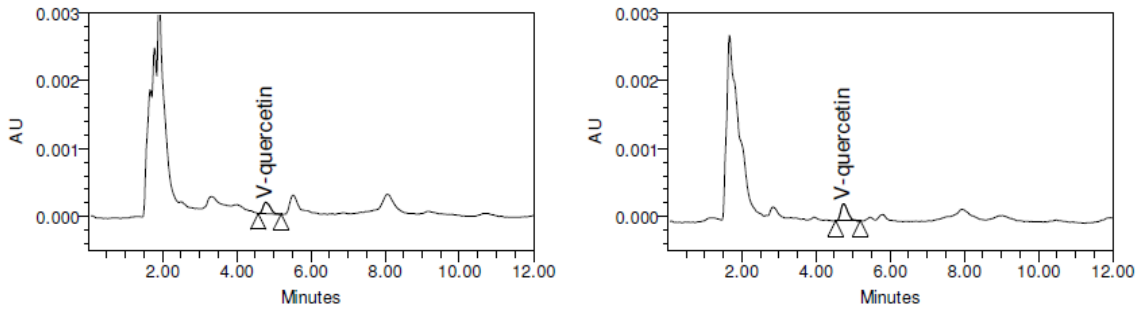


Fig.4. Representative chromatograms of plasma sample containing HOBQOV complex obtained at 1 hour after ip administration of 100 mg HOBQOV complex/ kg b.w. in rats.

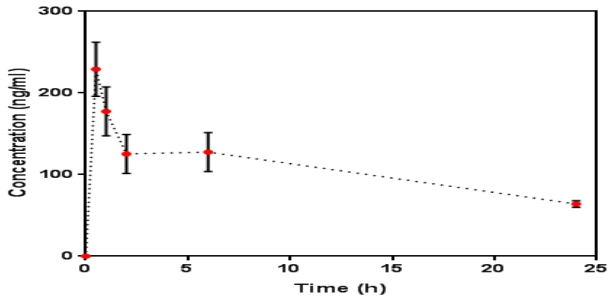


Figure 5. Comparative plasma concentration time profile for HOBQOV complex

Pharmacokinetic analysis

HOBQOV complex NC analysis

The pharmacokinetic parameters estimated by the NC methods are presented in table 2.

Table 2. Pharmacokinetic parameters calculated by the NC methods

Parameter	Value
$t_{½}$ (h)	21
MRT (h)	29
Cl_T (ml\min)	3,11
Vd (L)	94, 74
AUC_{0-24} (ng/mL*h)	2544.5

HOBQOV complex C analysis

Data fitting was performed for the monocompartmental, bicompartmental and tricompartmental models with immediate or delayed absorption. In Fig. 6 are presented the fitting results of the experimental data among each model statistics.

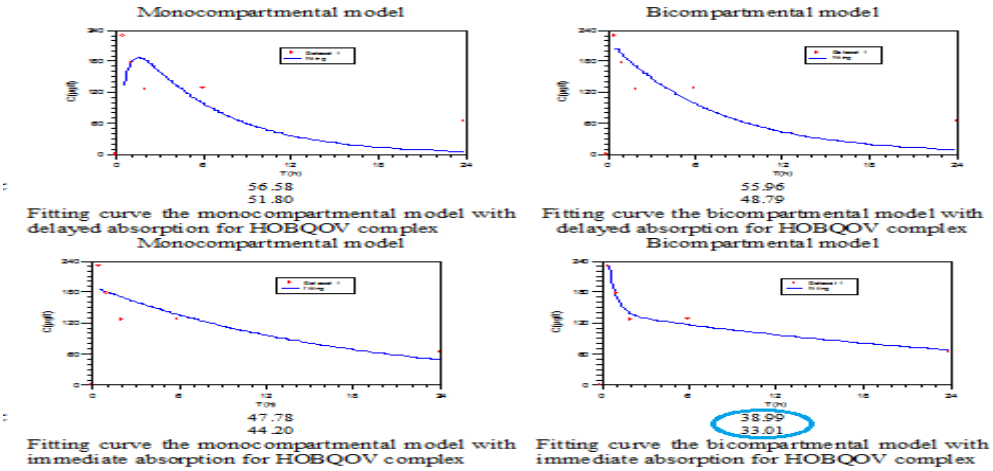


Fig.6. Fitting results of the experimental data

Statistic methods AIC and SC (with minimum values) pointed out the bicompartmental model with immediate absorption for HOBQOV complex as an optimum PK model.

The proposed bi-compartmental model that describes the HOBQOV pharmacokinetics after a single dose with intraperitoneal administration is described in Fig.7.

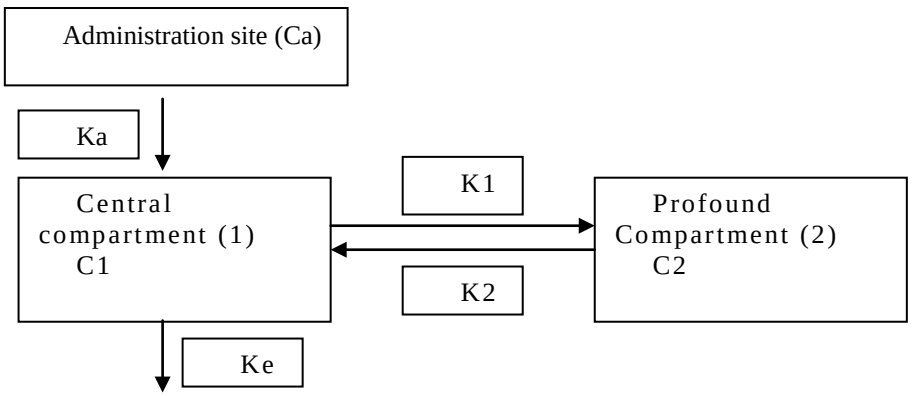


Fig.7.Proposed pharmacokinetic model for HOBQOV complex

DOI: 10.12865/CHSJ.40.04.06

The pharmacokinetic parameters estimated by the C methods are presented in table 3.

Table 3. Pharmacokinetic parameters calculated by the C methods

Parameter		Value
Cmax (ng/ml)		292
Tmax (h)		0.16
Macroconstants (h^{-1})	ka	1.84
	ke	0.74
Microconstants (h^{-1})	k12	16.92
	k21	0.75

Discussions

The data fitting for the bi-compartmental model with immediate absorption suggest that HOBQOV complex concentrations at the administration moment are zero in the body compartments and at the administration site. The complex appears to be immediately absorbed from the administration site (process characterized by the estimated apparent absorption constant ka and by the half time of absorption calculated as t1/2abs= ln2/Ka of 0.37 h). The distribution process from the central compartment in the profound compartment (process characterized by the global apparent transfer rate constant K calculated as K=k12/k/21, of 22.56) shows a cumulative trend for HOBQOV complex in the profound compartment. The elimination from the central compartment is the main process that contributes to the decrease of plasma concentration probably with the dissociation of the complex.

We can assume that HOBQOV plasma concentrations result from an equilibrium process between absorption, distribution and elimination. Elimination appears to be the main process that contributes to the concentration decrease of complex in the organism. In this context HOBQOV concentration from the central compartment decreases by 2 exponential processes, represented in Fig.8. The first one may be correlated with the distribution in the deep compartment in the first 2 hours after the administration. The second process may be correlated to elimination from, the central compartment.

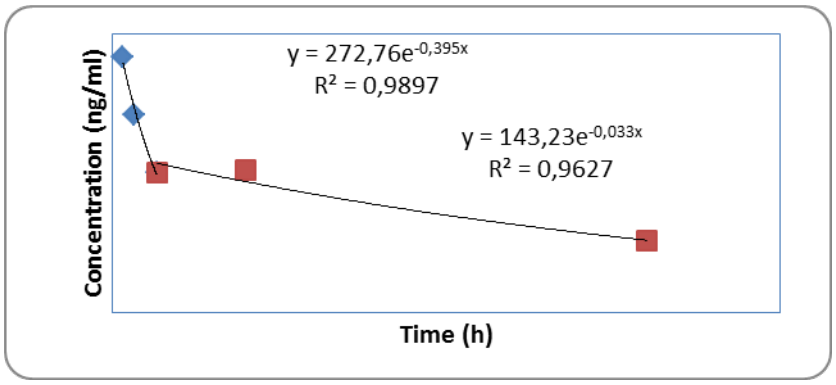


Fig.8. Biphasic decrease of HOBQOV complex plasma concentration

Conclusions

A HPLC method for the quantification of HOBQOV complex from rat plasma samples was developed, validated and applied in a single dose PK study.

The HOBQOV complex pharmacokinetics following the intraperitoneal administration is described by a bicompartmental model and the plasma concentration decrease is described by two phases that might be correlated to the distribution and elimination processes. The distribution profile of the complex points out a cumulative effect described by the global transfer constant between the central and the deep compartment >22.

Acknowledgements

This paper was co-financed from the European Social Fund, through the Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/159/1.5/S/138907 "Excellence in scientific interdisciplinary research, doctoral and postdoctoral, in the economic, social and medical fields - EXCELIS", coordinator The Bucharest University of Economic Studies.

References

1. World Health Organization (WHO). Country and regional data on diabetes. Available at http://www.who.int/diabetes/facts/world_figures/en

2. Montilla S, Marchesini G, Sammarco A, Trotta MP, Siviero PD, Tomino C, Melchiorri D, Pani L; AIFA Anti-diabetics Monitoring Group. Drug utilization, safety, and effectiveness of exenatide, sitagliptin, and vildagliptin for type 2 diabetes in the real world: Data from the Italian AIFA Anti-diabetics Monitoring Registry.Nutr Metab Cardiovasc Dis. 2014 Oct 6. pii: S0939-4753(14)00259-2. doi: 10.1016/j.numecd.2014.07.014. [Epub ahead of print]
3. Sakurai H, Katoh A, Kiss T, Jakusch T, Hattori M. Metallo-allixinate complexes with anti-diabetic and anti-metabolic syndrome activities. Metallomics. 2010 Oct;2(10):670-82.
4. Levina A, Lay PA. Metal-based anti-diabetic drugs: advances and challenges. Dalton Trans. 2011 Nov 28;40(44):11675-86.
5. Sakurai H. Tsuchiya K. Nukatsuka M. Sofue M. Kawada J, Insulin-like effect of vanadyl ion on streptozotocin-induced diabetic rats. J Endicronol 1990, 126:451-459
6. Thompson KH, Lichter J, LeBel C, Scaife MC, McNeill JH, Orvig C. Vanadium treatment of type 2 diabetes: a view to the future. J Inorg Biochem. 2009 Apr;103(4).
7. Goldwaser I, Gefel D, Gershonov E, et al. Insulin-like effects of vanadium; basic and clinical implications. J Inorg Biochem. 2000;80:21–5.
8. Badea M. Olar R. Marinescu D. Uivarosi V. Aldea V. Nicolescu TO, Thermal stability of new vanadyl complexes with flavonoid derivatives as potential insulin-mimetic agents. J Therm Anal Calorim 2010, 99:823-827
9. Margina, D.; Velescu, B.; Uivarosi, V.; Aldea, V.; Negres, S.; Mitrea, N., Evaluation of insulin-mimetic activity of new vanadyl-flavonoid complexes in alloxan-induced diabetes. FEBS JOURNAL, 277, p. 61, 2010
10. Velescu BS, Uivarosi V, Negres S. Effect of di-μ-hydroxo-bis(quercetinatooxovanadium(IV)) complex on alloxan-induced diabetic rats. Farmacia 2012, 60(5), 696-710
11. Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Control AC antioxidant status in diabetic rat lenses. J Biosci 30(2):221–230
12. 14. FDA. Food and Drug Administration,Center for Drug Evaluation and Research (CDER): Bioanalytical Method Validation- Guidance for Industry. 2001.
13. Mircioiu I., Aldea A., Anuta V., Neagu O., Miron D., Radulescu F., Enache F. Internal standard versus external standard in bioanalytical assay for bioequivalence studies – Farmacia, 2007, LV, 5, 569-580

Corresponding Author: Valentina Uivarosi, "Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Department General and Inorganic Chemistry, 6 Traian Vuia Str., 020956, Bucharest, Romania; tel.: +40740593237; e-mail: valentina_uivarosi@yahoo.com

 DOI: 10.12865/CHSJ.40.04.06