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PHARMACODYNAMIC AND PHARMACOKINETIC STUDIES ON NOVEL VANADYL CHRYSIN COMPLEX

Bruno Stefan VELESCU[1], Valentina UIVAROŞI[2], Valentina ANUŢA[3], Anca BUZESCU[1], Simona NEGREŞ[1]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy,
"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Department of Inorganic Chemistry, Faculty of Pharmacy,
"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[3]Department of Physical chemistry, Faculty of Pharmacy,
"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT. A novel complex with general formula $VO(chrysin)_2 \cdot 4H_2O$ was studied in order to evaluate its' pharmacodynamic action, and its' pharmacokinetic properties. The hypoglycemic activity of the complex was evaluated in an alloxan diabetic rat model, and compared to chrysin and vanadyl sulphate. The pharmacokinetic properties were studied following a single dose administration, in compartmental and non-compartmental analyses. The complex has a low toxicity and a low hypoglycemic effect ($p>0.05$) correlated with the pharmacokinetic parameters (volume of distribution, half time and micro-constants).

Keywords: vanadyl chrysin complex, pharmacodynamic action, pharmacokinetic properties, hypoglycemic activity

INTRODUCTION

The use of inorganic compounds in therapy is an increasing field of research motivated mainly by the success of cisplatin in chemotherapy.

Such potential inorganic drugs are vanadium compounds used in the treatment of the type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes. Early studies started with simple inorganic vanadium(IV/V) salts which shown insulin-like effects, but these vanadium salts are poorly absorbed from the gastro-intestinal tract, therefore the finding of orally active insulinomimetic compounds have to be directed towards the improvement of bioavailability of such salts by complexation with less toxic low-molecular-weight ligands. Complexes of oxovanadium (IV) cation and flavonoid derivatives were recently developed. The entrapment of oxovanadium (IV) ion in complexes with natural ligands such as the above mentioned, is a premise for diminishing the major problems of toxicology of the vanadium compounds (decreased GI absorption and GI irritation) and increasing the oral absorption.

The hypoglycemic activity of the complex VO(chrysin)2·4H2O (fig. 1) was assessed in an alloxan induced diabetes rat model. The hypoglycemic activity was compared with vanadyl sulphate and chrysin as reference substances. The pharmacokinetic parameters were assessed in a single dose experimental study with extra-vascular administration. Plasma levels of the VO-chrysin were determined with a validated HPLC method. Pharmacokinetic analysis was performed using TOPFIT Ver2.0. software in Non Compartmental (NC) and Compartmental (C) methods.

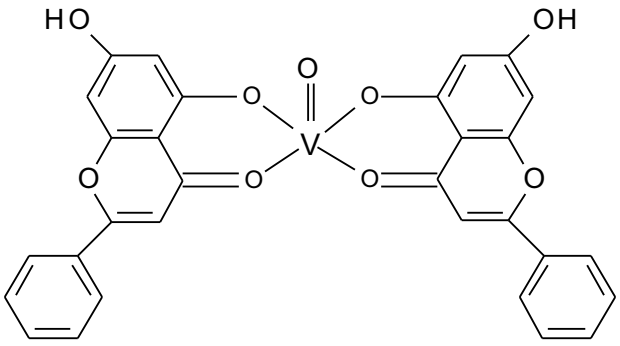

Fig. 1 Structure of vanadyl-chrysin complex

MATERIALS AND METHODS

Materials

Alloxan, chrysin, acetonitrile, trifluracetic acid, dimethylsulfoxide, diethyl-ether, hexane were purchased from Sigma Aldrich®.

Vanadyl-chrysin complex was synthesized according to a method described in a previous paper.

HPLC grade water was purified by a TKA-Genpure UV system. Statistical analysis was performed using StatSoft, Inc. (2004). STATISTICA (data analysis software system), version 7, www.statsoft.com. All results are expressed as mean ± standard deviation.

Experimental animals

Male Wistar and female rats weighing (129± 10 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 86/609/EEC of 24th November 1986, on the protection of animals used for experimental and other scientific purposes.

Diabetic animals

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate 13% (w/v) in a saline solution at a dose of 130 mg/kg body weight, after a fasten period of 12 hours. Blood samples were collected 48 hours later and glucose levels were determined to confirm the development of diabetes.

Venous glucose determinations were carried out using a commercial kit for glucose monitoring, BioLand G-423 glucometer, BioLand Technology LTD; blood samples from the tail vein of the rat were collected by puncture.

The diabetic animals were randomly separated into 4 groups of 6 rats:

- diabetic rats (D) treated with water 1mL/100 g b.w. for 5 days;
- diabetic rats treated with VO2+-chrysin complex (VO-ch) 0,8 mmol/kg b.w., aqueous suspension 0,8 mmol % for 5 days
- diabetic rats treated with chrysin (Ch) 0,16 mmol/kg b.w., aqueous suspension 0,16 mmol % for 5 days
- diabetic rats treated with vanadyl sulphate (V) 0,8 mmol/kg b.w., aqueous solution 0,8 mmol % for 5 days

Pharmacokinetics

Study design

26 female Wistar rats (137± 8 g) were randomly distributed in 5 groups of 5 animals 1 animal used as blank.

The animals received 100 mg/b.w. Vo-chrysin complex, by intraperitoneal injection.

Following the administration the animal groups were sacrificed at 30 min, 1h, 2h, 6h and 24h respectively. The blood was collected on Na2EDTA and was centrifuged; plasma samples were stored at -22ºC for HPLC analyses. Pharmacokinetic analysis was performed with TOPFIT Ver2.0. software.

HPLC assay

The HPLC method used a Waters Chromatographic system (Waters, Milford, MA, USA). The chromatographic conditions are presented in table 1:

Table 1

Chromatographic conditions of the HPLC assay

Parameter	Unit
Column	Kromasil 100-5C18, 150*4.6 mm
Temperature	25°C
Mobile phase	Solvent A: trifluoroacetic acid 0.1%
	Solvent B: acetonitril
Solvent A:Solvent B	30:70 v/v
Flow rate	1 ml/min
Detection	UV, 280 nm
Injection volume	100 µl
Run time	12 min

Plasma standards preparation

Standard plasma samples: Stock solution containing 250 µg/ml of VO-chrysin were made by dissolving 10,0 mg of VO-chrysin 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.

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RESULTS AND DISCUSSIONS

Hypoglycemic activity

Of the collectivity receiving alloxan, 72,42% were found to be diabetic (blood glucose > 200 mg/mL).

Daily blood level values were measured for the treated animals.

The mean blood glucose evolution is presented in the tables 2-4.

Table 2

Mean blood glucose evolution vs. basal and day 1 of treatment (24 hours after alloxan administration) for D group (%)

Day	D0	D1	D2	D3	D4	D5
Mean± SD (mg/dl)	86,50± 19,75	517,33± 111,68	457,00± 53,56	490,67± 64,38	470,33± 79,92	448,50± 97,42
Mean blood glucose evolution vs. basal (%)		498,07	428,32	467,24	443,74	418,50
Student test, paired values, 90% CI; p		<0,05	<0,05	<0,05	<0,05	<0,05
Mean blood glucose evolution vs. day 1 of treatment (%)			-11,66	-5,15	-9,09	-13,31
Student test, paired values, 90% CI; p			>0,05	>0,05	>0,05	>0,05

Table 3

Mean blood glucose evolution vs. basal and day 1 of treatment (24 hours after alloxan administration) for VO-ch group (%)

Day	D0	D1	D2	D3	D4	D5
Mean± SD (mg/dl)	100,75± 28,44	464,75± 140,13	457,33± 141,33	422,17± 134,29	375,83± 169,60	388,33± 231,72
Mean blood glucose evolution vs. basal (%)		361,29	353,93	319,02	273,04	285,44
Student test, paired values, 90% CI; p		<0,05	<0,05	<0,05	<0,05	<0,05
Mean blood glucose evolution vs. day 1 of treatment (%)			-1,60	-9,16	-19,13	-16,44
Student test, paired values, 90% CI; p			>0,05	>0,05	>0,05	>0,05

Table 4

Mean blood glucose evolution vs. basal and day 1 of treatment (24 hours after alloxan administration) for V group (%)

Day	D0	D1	D2	D3	D4	D5
Mean± SD (mg/dl)	99,33± 8,80	417,50± 69,96	471,17± 114,65	380,50± 56,83	370,50± 67,05	379,67± 57,74
Mean blood glucose evolution vs. basal (%)		320,30	374,33	283,05	272,99	282,21
Student test, paired values, 90% CI; p		<0,05	<0,05	<0,05	<0,05	<0,05
Mean blood glucose evolution vs. day 1 of treatment (%)			12,85	-8,86	-11,26	-9,06
Student test, paired values, 90% CI; p			>0,05	>0,05	>0,05	>0,05

Fig. 3 Calibration plot of the method

Table 5

Mean blood glucose evolution vs. basal and day 1 of treatment (24 hours after alloxan administration) for Ch group(%)

Day	D0	D1	D2	D3	D4	D5
Mean± SD (mg/dl)	122,00± 23,00	482,17± 184,33	450,50± 173,14	469,17± 162,82	492,50± 160,07	489,00± 180,36
Mean blood glucose evolution vs. basal (%)		295,22	269,26	284,56	303,69	300,82
Student test, paired values, 90% CI; p		<0,05	<0,05	<0,05	<0,05	<0,05
Mean blood glucose evolution vs. day 1 of treatment (%)			-6,57	-2,70	2,14	1,42
Student test, paired values, 90% CI; p			>0,05	>0,05	>0,05	>0,05

At the end of the experiment, by analyzing the data values, it can observed the glycemia decreased statistically significant compared to D group (ANOVA $p<0.05$), only for the animals within the group receiving vanadyl sulphate.

HPLC validation

Validation of the analytical method:

For the plasma quantification of VO-chrysin, a chromatographic method was developed and validated. A representative chromatogram is presented in fig 2. The method was validated in accordance with international regulations.

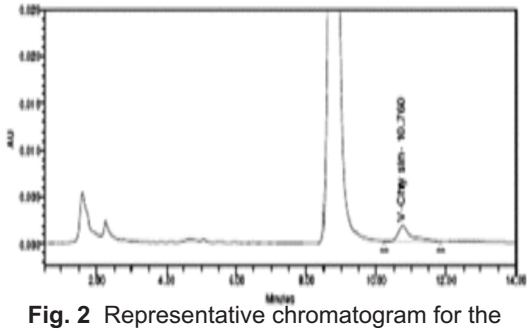

Fig. 2 Representative chromatogram for the plasma analyses of VO-chrysin

The calibration curve was linear in the range 0,01-50 µg/mL (r>0,9999); the lower limit of quantification (LLOQ) was 0,01 µ/mL (N = 6) (Fig. 3)

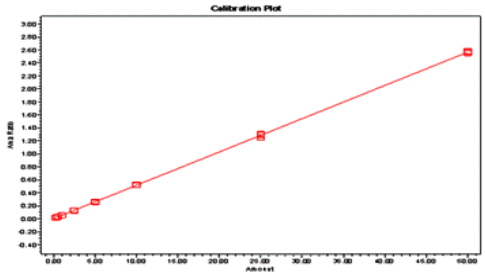

Fig. 3 Calibration plot of the method

For LLOQ concentration, the precision (characterized by the relative standard deviation) was 2,13%, and accuracy (defined as the deviation between the true and the measured value expressed in percent) was -0,77%. The intra-assay precision and accuracy was estimated by analysing 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 6). The precision was better than 5% and the bias did not exceed 4 % at all concentration levels tested. The recovery from plasma was influenced by the protein plasma binding of Vo-chrysin (77 % mean recovery); nevertheless the value of the recovery is appropriate for the determination of plasma level concentrations of the analyte.

Table 6

Intra-assay accuracy and precision of the method

Concentration (µg/mL)		Accuracy (%)	RSD (%)
Added	Measured		
30	29,9	-0,41	2,6
7,5	7,7	2,48	4,3
0,75	0,8	3,1	3,9

The concentration changes related to the nominal concentration were less than 15%, indicating no significant substance loss during the study

Plasma concentrations of VO-chrysin complex, at the sampling moments, are shown in table 7 and figure 4:

Table 7

Mean plasma concentration of VO-chrysin complex

Sampling time (h)	Plasma concentration (µg/mL)
0	0
0,50	0,48 ±0,58
1,00	0,37 ±0,21
2,00	0,23 ±0,06
6,00	0,05 ±0,05
24,00	0,01 ±0,01

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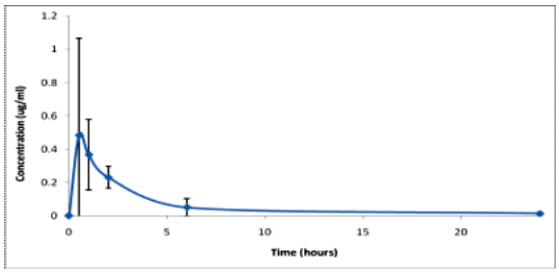

Fig. 4 Plasma concentration time profile for VO-chrysin complex

Pharmacokinetics

NC analysis

The pharmacokinetic parameters calculated by the NC methods are presented in table 6.

Table 6

Pharmacokinetic parameters calculated by the NC methods

Parameter	Value
$t_{½}$	6,5 h
TMR	4,5 h
Cl_T	119 mL/min
Vd	66,4 L

C analysis

Akaike (AIC) criteria were used for accepting the pharmacokinetic model described by the complex. The model with the lowest AIC value is accepted.

VO-chrysin complex evolution in the rat organism is described by a bicompartmental model (table 7).

Table 7

AIC values for the compartmental models

	Monocompartmental model	Bicompartmental model
AIC	-30,5	-32,1

The proposed pharmacokinetic model for VO-chrysin complex is described in fig. 5.

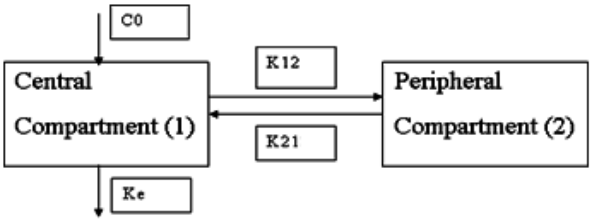

Fig. 5 Proposed pharmacokinetic model for VO-chrysin complex

The calculated micro- and macro- constants are presented in table 8.

Table 8

Calculated micro- and macro- constants for the bicompartmental model

Constant	1/h
K_{12}	0,19
K_{21}	1,04
Ke	0,45

The pharmacokinetic parameters of the novel VO-chrysin complex are presented in table 9.

Cmax	Tmax	K_{12}	K_{21}	Ke	TMR	Cl_T	Vd	$t_{½}$
0,576 µg/ml	0, 20 h	0,19 h^{-1}	1,04 h^{-1}	0,45 h^{-1}	4,6 h	119 ml\min	66,4 L	6,5 h

CONCLUSIONS

The calculated micro- and macro- constants for the VO-chrysin showed the complex tendency to a fast elimination.

In order to manifest its biologic activity, vanadium species have to interfere with different cytosolic enzymes. Targeting these intracellular enzymes is ensured by a proper pharmacokinetic profile. The tendency of VO-chrysin complex to return in the central compartment (K21/K12 ratio is approx 5.5), and the fast elimination (t1/2 6.5 h), indicates that the intracellular distribution of the complex is low; therefore the interaction with the cytosolic enzymes is diminished.

As a consequence, the inappropriate pharmacokinetic profile can lead to a low pharmacodynamic effect .

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