ТЕОРЕТИЧЕСКАЯ МЕДИЦИНА

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PHARMACOKINETICS AND ORAL BIOAVAILABILITY OF ANDROGRAPHOLIDE FROM KAN JANG TABLETS IN HUMANS

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Andrographis paniculata (AP) Nees is one of the most important medicinal plants, having been used in Chinese and Ayurvedic medicine for gastric disorders, colds, influenza and other infectious diseases [2, 6, 7, 8, 11, 18, 20]. The extract of AP standardized for its content of andrographolide and deoxyandrographolide and called 'Kan Jang', has been used extensively in Scandinavia for the last 20 years in treating the common cold. Several randomized placebo-controlled double blind clinical trials of Kan Jang tablets have shown that it has a preventive effect on the common cold and has the capacity to significantly shorten the duration of the ailment [4, 15, 13]. AP Nees has been demonstrated to be effective in the treatment of adult pharyngotonsillitis [19].

It has been shown in several animal studies that extracts of AP and its constituents, namely the diterpene lactone andrographolide, have anti-inflammatory [18, 6], anti-allergic [12], immuno-stimulatory [16] and antiviral activity [8, 14]. Recent findings indicate that the anti-inflammatory effects of Andrographis paniculata are possibly associated with the inhibition of the PAF-mediated inflammatory response [1] and the inhibition of nitric oxide synthesis in macrophages [9], but not with the inhibition of the biosynthesis of eicosanoides, as it is for NSAID [1]. This effect of andrographolide is also associated with the cardiovascular and anti-thrombotic activity of AP [3, 13, 21–25].

However, oral bioavailability and pharmacokinetic data of andrographolide have not yet been studied in either animals or humans. These data are extremely important not only for determining the right dosage regime, but also for eliminating the possibility of side effects due to overdosing. The aim of this study is to measure the rate of absorption, distribution and elimination of andrographolde (AND) in humans.

Materials and Methods

Kan Jang fixed combination tablets, Batch 88 32305, containing a fixed combination of 85 *mg* of extract of AP Nees (EX 20 396) and 11.6 *mg Acanthopanax senticosus*

siccum (Batch EX 20427) were formulated and supplied by Swedish Herbal Institute, Gothenburg.

The AND content was 5% in the extract and 4.25 *mg* per dosage form (tablets).

Pure AND supplied by Swedish Herbal Institute was used as analytical reference substance and was for purity by HPLC, GC-MS, ^{1}H-NMR, capillary electrophoresis (CE) and ^{13}C-NMR.

The data on AND were obtained from 16 healthy volunteers (seven males and nine females, mean age 39.8 years, range 23-48, mean weight 68.95 *kg*, no medication in the previous month) who took part in an open, single dose, randomised study. The Ethical Committee of the Armenian Drug Administration approved the study and the written consent to participate was obtained from the subjects. The subjects fasted for 10 hours. At 8 a.m., 2 hours before breakfast, each received four tablets of Kan Jang fixed combination (4 x 4.25 mg of AND) with 200 *ml* of water. Blood samples (9 *ml* each) were taken before administration and 0.5, 1.0, 1.5, 2, 3, 4, 6, and 8 hours thereafter. Blood was sampled directly in EDTA-coated tubes from vein puncture and the prepared plasma was stored at –20^{o}C until analysis.

Drug safety was rated based on physical examination, measurement of blood pressure, heart rate, laboratory examination of haematology (leukocytes, erythrocytes, haemoglobin, hematocrit, MCV, MCH), biochemistry (glucose, creatinine, gamma GT, sodium, potassium) and documentation of adverse effects. Participants were asked about any adverse effects and the answers recorded by the clinical staff every hour after drug intake. The classification of the AE's was in accordance with EC-guideline 111/3445/91-EN: mild, moderate and severe; the causality of the study medication was determined as definite, probable, possible, unlikely, not related and not possible to judge.

Instrumentation

HEWLETT PACKARD Capillary Electrophoresis System analyzes samples:

Instrumentation:	HP High Performance Capillary Electrophoresis System, consisting of HP^{3D}CE, HP KAYAK XA, HP Laser Jet 4000 printer.
Data Collection:	HP ChemStation for CE systems
Statistical analysis:	Prism software, version 2.0, GraphPad Software Inc.USA, 1996

Experimental conditions

Mobile phase	Borate buffer pH 9.3 (20 *mM*) / Methanol = 90/10 (*v/v*)	
Capillary	HP-Part number - G 1600-61232	
	Total length	64.5 *cm*
	Effective length	56 *cm*
	i.d.	50 *µm*
	Optical path length	150 *µm* (*Bubble factor – 3*)
Injection	50 *mbar/4 sec*	
Temperature	30 ^{o}*C*	
Voltage	15 *kV*	
Detection	target signal 229/8 *nm*, reference 500/100 *nm*	
	signal 195/8 *nm*, reference 500/100 *nm*	
	signal 229/8 *nm*, reference 271/20 *nm*	

Sample preparation

3 *ml* of methanol was added to 2 ml of blood plasma; and vortex and proteins were precipitated for 15 *min* at 4°C by centrifugation at 3000 *rpm*. Supernatant was removed and diluted with 20 *ml* of water. Samples were purified by solid extraction using LC-18 tubes. Samples were applied to Supelclean LC-18 tubes (3 *ml*, pre-washed with 5 *ml* of methanol and 10 *ml* of water) followed by elution separately with 10 *ml* of water and 10 *ml* of methanol-water mixture, 9:1 (separately). The methanol-water solution was evaporated to dryness using a vacuum rotary evaporator. The residue was dissolved in 100 *ul* of mobile phase: 20 *mM* borate buffer pH 9.3 - methanol, 90:10, *v/v* and used fresh for injection to HPCE. Statistical analysis was performed using GraphPad PRISM software, version 2.0, 1996, GraphPad Software, Inc. USA

The following model of independent pharmacokinetic parameters was calculated using the TOPFIT, version 1.1 (Godecke AG/Schering G/Thomae GmbH) program.

Pharmacokinetic parameters

C_{max}, *ng/ml*	the maximum concentration was taken direct from the concentration course
t_{max}, *h*	time to achieve C_{max}
K_{el}, h^{-1}	elimination rate constant calculated by log/linear regression of the concentration/time data using 4 data points (terminal slope)
$t_{1/2}$, h	elimination half-life (0.693/*kel*)
$AUC_{0-\infty}$, *ng • h/ml*	area under the curve after extrapolation from time x to infinity, where x is the last time point with a concentration above the lower limit of quantification AUC (0-t) + c_x/K_{el}
Cl_t, *ml/min*	total clearance = F • dose / AUC
MRT, h	mean residence time = AUMC/AUC

Dose , *mg* for the calculation of AND dose, the actual concentrations were used as determined analytically.

K_{abs}, h^{-1}	absorption rate constant
$t_{1/2\ ka}$, *h*	absorption half-life
V_d , l	apparent volume of distribution (F • dose / C_o)
C_o, *ng/ml*	initial drug concentration
A , *ng/ml*	intercept of monoexponential α line with ordinate
α , h^{-1}	slope of monoexponential distribution line (hybrid constant)
$t_{1/2\alpha}$, *h*	distribution half-life
β , h^{-1}	slope of monoexponential declining line (hybrid constant)
B, *ng/ml*	intercept of back-exponential monoexponential declining line with ordinate
C_0, *ng/ml*	intercept of K_a slope with ordinate.

Results and Discussion

The course of the mean plasma concentration, estimated in a study of human volunteers, is shown in fig.1. and table1. It has been found that pharmacokinetics of AND is highly variable in individuals. Pharmacokinetics of AND using mean values of concentra-

tions is well characterized by two-compartment model and three-exponential equation [17]:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} - C_o e^{-ka\,t}$$

Or

$$C_t = 1107.0e^{-0.8934t} + 98.49e^{-0.104t} - 2091.9e^{-1.33\,t}$$

Accuracy of this model was checked particularly by calculation of theoretical concentration after 3 hours of drug administration. Theoretically calculated concentration was 138.2 *ng/ml* and experimentally found – 141.7±20 *ng/ml*. Thus, it seems that pharmacokinetic parameters of AND obtained from mean concentrations are more accurate than mean values of pharmacokinetic constants of individuals, which are highly variable.

AND is quickly absorbed in blood after oral administration of Kan Jang fixed combination, absorption half-life $T_{1/2ka}$ is about 20 *min*. The maximal concentration of AND in the blood was found to be 1.6 hours after administering Kan Jang. At four volunteers distribution of andrographolide in tissues occurs comparatively quickly and the data nicely fit a one-compartment model using the TOPFIT program. At other 12 subjects the drug slowly distributed in body organs and tissues, mainly in 1.5-3 hours after administration. In these cases the pharmacokinetics of andrographolide is very well described by two-compartment model (tables 2 and 3).

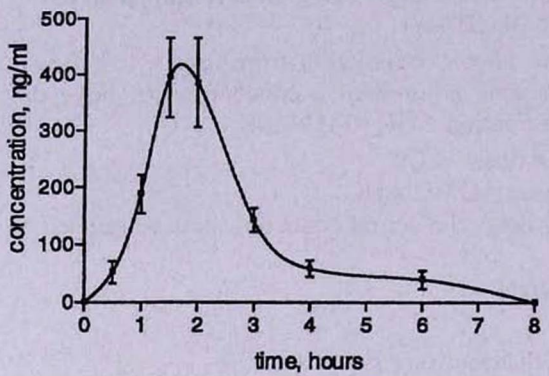

Fig. 1. Plasma concentration of AND vs time after oral administration of Kan Jang (four tablets, 20 *mg* AND) to humans (mean± SEM, n=16)

Table 1

Concentration of andrographolide in blood plasma of healthy subjects (*n=16*) Single dose, oral administration of four Kan Jang fixed combination tablets

Time, *hrs*	Mean	SEM	SD
0.000	0.000	0.000	0.000
0.500	52.14495	18.89454	75.57814
1.000	187.3985	33.67654	134.7061
1.500	393.3676	70.46471	281.8589
2.000	384.3903	79.12016	316.4806
3.000	141.7496	20.64165	82.56658
4.000	57.92042	14.02963	56.11852
6.000	38.89634	15.66465	62.65858
8.000	0.000	0.000	0.000

Table 2

Individual pharmacokinetic parameters of AND in every human subject (n=15*)

Volunteer No	$t_{1/2\beta}$, h	Cl_t, ml/min	V_d, l	β, h-1	K_a, h^{-1}	t_{MAX}, h	MRT, h	$AUC_{0-\infty}$, ng● h/ml	C_0=B	A	$t_{1/2\alpha}$, h	Compartment model
1	1,57	180	25.15	0.44	1.08	1.69	3.2	1840	118.2	-	-	1
2	1.48	201.3	90.1	0.47	0.95	1.41	2.68	475.3	419	-	-	1
3	2.37	215.5	44.25	0.29	0.99	1.18	4.01	1547	1013	-	-	1
5	0.77	564	37.5	0.9	0.11	1.22	2.00	590.5	696.1	-	-	1
6	1.2	336.1	34.4	0.59	1.11	1.44	2.54	992	926.4	1990	0.65	2
7	0.9	284.1	21.58	0.78	1.34	1.07	2.45	1173	209.8	1572	0.52	2
8	14.99	307.4	12.42	0.05	0.53	0.51	20.37	1084	47	1589	0.47	2
9	1.81	177	27.79	0.38	1.22	1.76	2.82	1883	533	2164	0.57	2
10	4.75	377.4	155.2	0.15	2.04	1.23	5.69	883.3	90.85	1205	0.92	2
11	3.52	179.6	54.7	0.2	0.8	1.76	4.45	1856	271.7	1346	0.4	2
12	3.48	190	57.24	0.21	1.74	1.5	3.93	1754	225.2	2171	0.15	2
13	4.97	364.7	157	0.14	0.82	1.6	5.73	914	90.2	558.8	0.22	2
14	11.87	195.3	12.31	0.06	0.9	1.65	13.77	1707	77.07	1625	0.32	2
15	3.88	222.4	61.19	0.22	1.28	1.25	3.84	1499	238.2	1559	0.53	2
16	1.54	274.9	36.53	0.45	4.4	1.2	1.9	1213	250	3865	0.16	2
Mean	**3.940**	**271.3**	**55.16**	**0.355**	**1.29**	**1.365**	**5.292**	**1294**	**347.0**	**1786**	**0.44**	
SD	4.126	106.8	45.74	0.253	0.97	0.328	5.079	469.3	310.3	827.9	0.23	
SE	1.065	27.58	11.81	0.065	0.25	0.084	1.311	121.2	80.13	249.6	0.06	

* - High deviations of concentrations of AND in volunteer No 4 did not allow to calculate pharmacokinetic parameters.

Table 3

Pharmacokinetic parameters of AND in healthy human subjects calculated using the mean values of individual parameters and mean concentrations of AND

PARAMETERS	Mean values of model independent pharmacokinetic parameters calculated for individuals	Two-compartment model pharmacokinetic parameters calculated using mean values of concentrations of Andrographolide
Cl_t, *ml/min*	271.30±27.58	254.600
V_d, l	55.100±11.81	140.000
β, h^{-1}	0.355±0.06	0.104
K_a, h^{-1}	1.290±0.25	1.330
t_{max} h	1.365±0.08	1.450
MRT, h	5.292±1.31	6.386
$AUC_{0-\infty}$, ng● h/ml	1294.000±121.2	1309.000
t_s, h	3.940±1,06	6.670

Intensive elimination of AND began 3-4 hours after drug administration and at the 8th hour was not detected in the blood. In some cases it happened 4 hours after drug administration.

It might be suggested that high individual variability in pharmacokinetics of andrographolide, especially in the phase of distribution, is due to different extent of bounding with blood proteins.

In addition, andrographolide intensively metabolized, it was shown in rats. Since, in pharmacokinetics human subjects commonly are divided into "slowly" and "quickly" drug metabolizing subjects, it may be assumed that individual variability in distribution and elimination rates is consequence of various metabolizing rate of andrographolide. On the contrary, when drug metabolism and its bounding with blood proteins are not intensive the absorption of drug docs not vary substantially in individuals. Absorption rate constant and t_{max} in 13 subjects was approximately the same.

Thus, pharmacokinetics of AND is characterized by high absorption from gastrointestinal tract into the blood. Then it is intensively bounded with blood proteins and redistributed between blood and tissues within 1-2 hours. Elimination half time is in range of 2-7 hours. The main rout of elimination is metabolism.

In the view of the facts that for therapeutic purposes, 12 tablets of Kan Jang fixed combination are administered per day (i.e. four tablets given 3 times a day), the effective plasma level of AND can be predicted from computer fit data. Using equations for calculating the average drug concentration in the blood at multiple doses [17], the dose of AND in Kan Jang fixed combination 4tab. daily/70*kg* (intervals 4*hrs*) using the single dose data were obtained (table 4,fig.2).

Table 4

Calculated maximal and minimal concentrations of AND (*ng/ml*) after multiple oral administration of Kan Jang

N	1	2	3	4	5	6	7	8
C^n max	578.5	876.8	1200	1320	1339	1341.4	1342	1342.1
C_{pn}	-	590	637.2	649.9	654.5	655	655.53	655.6
C^n min	231.4	460.5	536.8	552.9	556.7	558.3	558.6	558.7

C^n_{max} - maximum drug concentration in the blood for multiple doses at a steady state, *ng/ml*
C^n_{min} - minimum drug concentration in the blood for multiple doses at a steady state, *ng/ml*
C_{SS} - mean (average) drug concentration in the blood for multiple doses at a steady state, *ng/ml*
C_{pn} - drug concentration in the blood after n^{th} dose, ng/ml
n - number of administrations

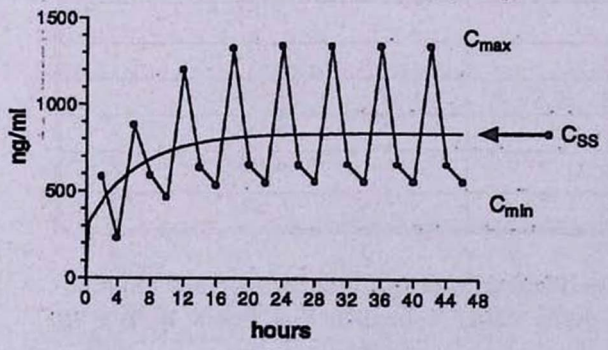

Fig.2. Steady-state simulation of AND plasma concentration after oral administration of Kan Jang (four tablets, 20 *mg*/70 *kg* using the single dose data)

Figure 2 shows that the steady state concentration of AND, (RSD is 3.74% after every new administration) will be established after 3 administrations of four tablets at 4 *hrs* intervals – that is on the first day of the treatment.

The calculated steady state plasma concentration of AND for multiple doses of Kan Jang fixed combination (after the normal therapeutic dose regimen, 3 x 4 tablets/day) was approximately 660 *ng/ml* (approx. 1.9 *μM*). That is enough to reveal any anti-PAF effect, particularly after drug uptake, when the concentration of AND in blood is about 1342 *ng/ml* (approx. 3.8 *μM*), while for anti-PAF effect EC_{50} – 5 *μM* [1].

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ԱՆԴՐՈԳՐԱՖՈԼԻԴԻ ՖԱՐՄԱԿՈԿԻՆԵՏԻԿԱՆ ԵՎ ԿԵՆՍԱՍՏՅԵԼԻՈՒԹՅՈՒՆԸ ՄԱՐԴԿԱՆՑ ՄՈՏ KAN JANG ԴԵՂԱՀԱԲԵՐԻ ԸՆԴՈՒՆՈՒՄԻՑ ՀԵՏՈ

Է.Ս. Գաբրիելյան, Գ.Վ. Մամիկոնյան, Ա.Գ.Փանոսյան, Ա.Ս.Հովհաննիսյան, Հ.Գ. Աբրահամյան, Գ. Վիկման

16 կամավորների մոտ հետազոտվել է անդրոգրաֆոլիդի, *Andrographis paniculata* բույսի ակտիվ նյութի ֆարմակոկինետիկան *Andrographis paniculata* թուրմ պարունակող *Kan Jang* հաբերի միանվագ (թերապևտիկ դեղաչափ) ընդունումից հետո: Անդրոգրաֆոլիդի քանակական որոշումը իրականացվել է բարձրաէֆեկտիվ կապիլյար էլեկտրոֆորեզի մեթոդով: Հետազոտությունները ցույց են տվել, որ անդրոգրաֆոլիդի մաքսիմալ քանակը արյան պլազմայում հայտնաբերվում է հաբերն ընդունելուց 1,5-2,0 ժամ հետո և կազմում է 393 *նգ/մլ* (1.12 *μM*): Կիսադուրսմման ծամանակը կազմում է 3,94 ժամ: Կատարված հաշվարկները ցույց են տվել, որ օրական 3x4 հաբ ընդունելուց հետո անդրոգրաֆոլիդի հավասարակշիռ խտությունը արյան պլազմայում կարող է կազմել 660 *նգ/մլ* (1.9 *μM*): Այդ քանակը բավարար է հակա-PAF ազդեցություն սպանալու համար:

ФАРМАКОКИНЕТИКА И БИОДОСТУПНОСТЬ АНДРОГРАФОЛИДА У ЛЮДЕЙ ПОСЛЕ ПРИЕМА ТАБЛЕТОК KAN JANG

Э.С.Габриэлян, Г.В.Мамиконян, А.Г.Паносян, А.С.Оганесян, А. Г.Абраамян, Г. Викман

Исследована фармакокинетика андрографолида, активного ингредиента растения *Andrographis paniculata* у добровольцев после приема таблеток Kan Jang, содержаших стандартизированный экстракт *Andrographis paniculata*. Количественное определение андрографолида в плазме крови проводили методом высокоэффективного капиллярного электрофореза.

Установлено, что после однократного введения таблеток в терапевтической дозе его концентрация в крови достигает максимума через 1,5–2,0 *ч* и составляет примерно 393 *ng/ml* (1.12 *μM*). Период полувыведения и среднее время удерживания андрографолида составляют 3,94 и 5,29 часов соответственно.

Показано, что возможная равновесная концентрация андрографолида в крови после многократного введения таблеток (3 x 4 табл. в день) может быть примерно 660 *нг/мл* (примерно 1.9 μM). Данная концентрация является достаточной для проявления анти-PAF эффекта, особенно если учесть, что при многократном приёме равновесная максимальная концентрация андрографолида составляет 1342 *нг/мл* (примерно 3.8 *μM*), тогда как EC_{50} для анти-PAF эффекта – 5 *μM*.

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