Document 05

V-411

Therapeutical Experimentation Report

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V-411
Therapeutical Experimentation Report

5.1. ANIMAL TOXICITY AND SAFETY

These studies were carried out in China and were an integral part of the NDA submitted in that country.

The acute and chronic toxicity studies were performed by Xin Xing Pharmaceuticals Ltd. in the Chinese Academy of Military Medical Sciences between 1994 and 1995.

5.1.1. Acute Toxicity Studies

5.1.1.1. Oral LD50 study

The fifty percent oral lethal dose (LD50) was determined using seventy two (72) CD-1 mice sub-divided into 6 dosage groups, 12 mice per group. The vital organs of autopsied animals that had died during the course of study were examined macroscopically.

It was only possible to induce death following administration of very high doses of V-411. No clinical or macroscopic examination of the vital organs revealed any organ specific cause of death. The animals died from the effects of mechanical abdominal obstruction due to excessive amounts of drug in their intestines.

The results are given in the table and figure below. The death rate has been correlated with the average of the total dose given per group, and the average dose as a function of body weight.

Table 5-1 Results of oral LD50 experiments

Dose Mg	Dosage/ Body Weight (mg/kg)	Log Dosage (X)	No.of Death	Death Rate (P)	Probability Unit (Y)	Weighted Factor (W)	Group Factor (R)	Unit of death rate probability (R*Y)
1300.000	70000.0	4.8451	12	100.00	7.460	0.161	5	37.300
798.000	42000.0	4.6232	10	83.33	5.954	0.454	3	17.862
478.000	25200.0	4.4014	7	58.33	5.202	0.627	1	5.202
287.280	15120.0	4.1796	5	41.67	4.798	0.300	-1	-4.798
172.368	9072.0	3.9577	1	8.33	3.595	0.300	-3	-10.785
103.421	5443.2	3.7359	0	0.00	2.540	0.161	-2	-12.700
		Mean=3.290			**Mean=4.925**	**$\sum$W=2.003**		**$\sum$(R*Y)=32.081**

Calculations used to derive the LD 50, 95% confidence limits, and the slope of regression are given below.

(1) Regression Slope:

$$b = \frac{6 \bullet \sum (Y \bullet R)}{i \bullet k(k^2 - 1)} = 4.132$$

Whereas: b is regression slope
Y*R is Death Rate Probability Unit
i is the differences between log doses, which in this case is 0.2219
k is number of groups involved, which in this case is 6

(2) LD 50:

$$LD_{50} = AntiLog[\bar{x} + \frac{1}{b}(5 - \bar{y})] = 20354.91$$

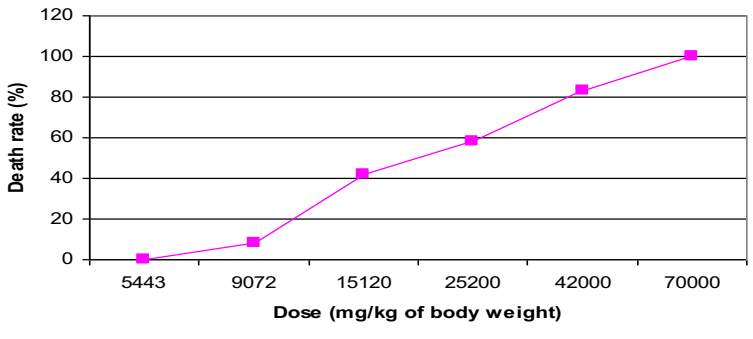
(3) L95 (The 95% Confidence Limit of LD 50):

$$L95 = LD50 \pm 4.5 \bullet LD50 \bullet \frac{1}{b \bullet \sqrt{n \sum w}} = 20354.91 \pm 4521.97 mg/kg$$

Whereas: n is number of animals per group

The calculated LD50 correlated precisely with what can be deduced from the graphic display in figure 5-1.

Figure 5-1 Oral lethal dose curve in mice



Therapeutic Index

The oral V-411 LD 50 dose in mice was found to be 20,354 mg/kg (± 4,521 mg/kg 95% confidence limits). If it is assumed that the LD 50 is the maximum acceptable toxic dose (TD 50), then the therapeutic index is derived by dividing the LD 50 by the 50% effective dose (ED50), which gives a TI in excess of 10,000.

Acute oral LD_{50} in mice	**20 grams/ kg**
Therapeutic Index in mice	**109000**
Dose equivalent for 75 kg man	**1.5 kilograms**

5.1.2 Chronic Toxicity Studies

The logistics of these studies were the same as above (5.1).

5.1.2.1 Study design

Two animal species were tested, rat and dog. Three hundred and twenty (320) Sprague Dawley rats, and eighty (80) Beagle dogs, were allocated to one of four oral treatment groups by randomization, consisting of one of three V-41 I dosage levels or placebo. The drug was given orally, daily for 30 weeks (270 days). The dosage levels were between 500 and 2000 times higher than the effective dose in mice. This study was doubleblind.

The animals were housed in an approved, supervised institutional animal house facility. Regularly cleaned, nourished, watered and cared for. They were supervised by the institute’s medical and technical staff.

5.1.2.2 Parameters tested

A broad spectrum of clinical, morbid anatomical and laboratory investigations were routinely, and regularly carried out to document any possible toxicity in the study. The results were recorded on institutional forms and later summarized and transferred to the study coordinators. The standard operating procedure for handling dying or dead animals was to perform a full autopsy, the report and it's findings were recorded in the study documentation. The institute has its own system of supervision and quality control. Dr. Xing provided the final interpretation of the results and report findings.

The parameters tested have been summarized in the following table. These investigations were done in both the rat and the dog studies.

Table 5-2 Clinical and laboratory parameters tested in chronic toxicity studies

Frequency of Observation	Clinical Parameters	Laboratory Parameters	Organ Size & Histology
Daily	General activity Eating Drinking Hair appearance		Heart Liver Spleen Kidneys
Weekly	Body Weight Heart rate		Lungs Intestine (small) Ovaries
Monthly	EKG	Hb & RBC WBC & Diff Platelet count Liver function tests* Renal function tests**	Testes Adrenal glands Stomach Colon Brain
		* AST & ALT ** BUN & CREA	*Macro- &* *Microscopic* *End of Study*

Footnote AST means aspartate amino transferase, ALT means alanine aminotransferase, BUN means blood urea nitrogen and CREA means plasma creatinine.

The results are expressed as the mean and standard deviation for each parameter, for each dose, for each animal group.

5.1.2.3 Results

5.1.2.3.1 Clinical Parameters.

No statistically significant changes in the clinical parameters tested were noted between the control and V-41 I treated groups, neither in the rat nor in the dogs. To exemplify this the mean +/- sd for body weight and heart rate are given in the following table.

The rats in the control and V-411 groups increased in body weight during the course of the study, by 173% and 177% respectively. These changes indicate that the animal husbandry was satisfactory. However the differences between the treatments is not significant and fall within the 95% confidence limits.

Baseline and end of study heart rate were virtually unchanged in both groups.

5.1.2.3.2 Hematological

No significant changes were noted between baseline and end of the hematological investigations, and no significant differences were noted between the control and V-41 I treated groups in both rats and dogs. The rat data is summarized in the following table.

5.1.2.3.3 Hepatic and Renal function

The only baseline-end of study increase worth noting was in the ALT changes, 3% for control rats, and 10% for V-411 treated rats. These and other changes between baseline and end of study hepatic and renal function tests were not significant, and no significant differences were noted between the control and V-41 I treated groups in both rats and dogs. The rat data is summarized in the following table.

5.1.3.2.4 Organ Size

No significant differences in organ size (weight) were noted between control and V-41 I treated groups in both rats and dogs. The extracts of the rat data are summarized in the following table. Complete listings of findings will be made available following translation.

The following tables provide detailed information as described above

Table 5-3: Body weight changes (Mean +/- SD)

Group	Sex	B.W Before Mean	SD	B.W. after (Mean)	SD
Control	Male	170.4	13.0	449.3	24.0
	Female	194.6	16.2	544.8	30.3
Low	Male	178.1	15.1	514.0	28.3
	Female	206.0	16.9	566.2	31.7
Medium	Male	195.4	16,2	546.4	30.4
	Female	191.2	15.9	538.2	29.9
High	Male	185.8	15.6	528.5	29.2
	Female	212.1	17.3	577.8	32.5

Table 5-4 Effects of VAI feeding on RBC counts (10000/cubic mm, Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	446.15	33.83	453.59	34.57	424.57	31.67	426.22	31.84
1	446.67	33.88	473.78	36.59	424.36	31.65	437.49	32.96
2	442.25	33.44	479.57	37.17	435.41	32.76	430.07	32.22
3	447.22	33.99	481.01	37.32	434.90	32.70	437.42	32.96
4	449.02	34.12	486.13	37.83	419.45	31.16	435.06	32.72
5	458.45	35.06	480.83	37.30	427.19	31.93	434.95	32.71
6	472.76	36.49	478.74	37.09	404.63	29.68	428.02	32.02
7	470.33	36.25	500.08	39.22	491.37	28.35	408.03	30.02

Table 5-5 Effects of VAI feeding on Hemoglobin (gram/100mL, Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	16.72	1.29	14.92	1.02	14.54	0.96	14.39	0.94
1	16.52	1.26	13.94	0.87	15.85	1.16	13.28	0.77
2	16.43	1.25	14.82	1.00	16.48	1.25	13.09	0.77
3	15.40	1.09	13.64	0.86	14.98	1.03	13.17	0.74
4	15.06	1.10	13.79	0.85	15.40	1.09	12.86	0.76
5	15.84	1.14	13.05	0.74	15.57	1.12	11.96	0.71
6	16.98	1.33	13.10	0.74	15.55	1.11	13.37	0.57
7	18.15	1.50	12.64	0.68	16.93	1.32	13.51	0.81

Table 5-6 Effects of VAI feeding on Platelets counts (1000/cubic mm, Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	33.12	4.17	31.16	4.10	32.68	4.04	32.12	3.73
1	34.43	4.36	31.17	4.12	31.53	3.87	30.43	3.48
2	34.33	4.35	32.07	4.24	31.44	3.85	30.01	3.41
3	34.01	4.30	32.08	4.24	30.82	3.76	30.77	3.53
4	33.83	4.27	31.40	4.14	32.24	3.97	28.97	3.26
5	34.09	4.31	31.56	4.16	32.95	4.08	28.20	3.14
6	32.70	4.10	31.37	4.13	31.79	3.91	27.23	3.42
7	32.88	4.13	31.17	4.10	30.87	3.77	26.99	2.96

Table 5-7 Effects of VAI feeding on WBC counts (1/cubic mm, Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	4983	497	4739	448	4516	403	4764	4534
1	5019	531	4599	442	4453	439	4971	497
2	5193	487	4644	466	4584	468	4952	487
3	5161	563	4380	462	4185	434	5034	504
4	5364	531	4348	432	4315	420	5003	593
5	5219	537	4221	445	4367	400	5028	502
6	5038	502	4264	406	4751	443	5141	536
7	5094	506	4427	430	5054	482	5300	530

Table 5-8 Effects of VAI feeding on Neutrophils (%, Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	38.5	3.7	38.9	3.8	41.6	4.3	41.0	4.2
1	37.8	3.8	39.8	4.1	37.9	4.1	38.2	4.2
2	36.9	3.5	41.6	4.1	36.9	4.0	36.6	3.7
3	38.1	3.8	42.1	3.9	38.6	4.0	33.3	3.6
4	35.2	3.9	39.6	4.0	40.6	3.8	34.5	3.3
5	34.8	3.5	40.5	3.9	41.9	4.3	34.9	3.4
6	35.0	3.3	41.2	4.1	40.8	4.1	37.8	3.6
7	33.8	3.6	39.4	4.2	39.2	3.8	39.1	3.9

Table 5-9 Effects of VAI feeding on Lymphocytes (%, Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	28.0	2.6	31.2	3.2	30.6	3.1	28.2	2.6
1	27.7	3.0	30.1	2.9	32.3	3.1	27.4	3.0
2	26.2	2.7	31.4	3.0	35.0	3.4	26.4	2.7
3	26.7	2.6	30.8	3.1	34.7	3.6	24.2	2.5
4	26.0	2.5	32.5	3.2	34.2	3.6	23.8	2.2
5	24.9	2.6	31.9	3.2	33.4	3.3	23.1	2.3
6	26.0	2.4	33.8	3.3	34.3	3.2	24.9	2.4
7	25.2	2.4	35.3	3.3	34.7	3.6	25.0	2.5

Table 5-10 Effects of VAI feeding on AST (micromol/L Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	37.13	3.73	37.78	3.86	36.33	3.57	38.96	4.09
1	38.09	3.69	38.90	3.88	34.28	3.43	40.45	3.88
2	40.12	3.90	40.70	3.84	37.52	3.73	38.17	3.82
3	41.41	4.03	41.60	4.01	37.89	3.85	36.79	3.64
4	41.76	4.20	41.61	4.35	34.99	3.50	36.92	3.83
5	43.60	4.09	42.14	4.32	35.94	3.52	36.76	3.73
6	43.11	4.61	40.39	4.22	33.49	3.47	35.50	3.56
7	43.22	4.05	42.87	3.97	35.53	3.43	34.87	3.732

Table 5-11 Effects of VAI feeding on ALT (micromol/L Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	107.6	10.8	99.9	9.3	107.6	10.8	111.9	11.7
1	103.0	10.6	102.7	10.3	106.6	10.2	120.7	11.7
2	111.3	10.5	109.7	10.4	107.2	10.5	120.4	12.1
3	102.2	10.3	114.6	11.0	110.1	11.0	116.3	12.0
4	99.7	10.1	110.6	11.8	104.6	11.1	108.3	11.0
5	102.8	10.5	104.3	10.3	107.6	10.5	114.0	10.7
6	106.6	10.7	103.9	10.9	100.0	10.4	119.0	11.2
7	110.8	10.8	105.4	10.1	108.4	10.1	123.4	11.9

Table 5-12 Effects of VAI feeding on BUN (micromol/L Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	20.01	2.00	20.55	2.11	20.01	2.00	20.13	2.03
1	19.48	1.91	20.98	2.06	20.83	1.95	19.83	2.09
2	19.77	1.95	20.11	2.08	21.26	2.12	20.60	1.89
3	21.28	2.17	18.39	1.90	22.23	2.16	19.63	2.05
4	21.95	2.23	17.74	1.74	21.98	2.25	20.68	1.93
5	21.26	2.26	18.12	1.64	23.33	2.27	21.79	2.14
6	21.32	2.28	16.33	1.81	22.85	2.22	22.07	2.31
7	22.63	2.24	16.67	1.68	22.10	2.14	22.56	2.35

Table 5-13 Effects of VAI feeding on CREA (micromol/L Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	145.30	14.06	140.45	13.09	152.71	15.54	154.39	15.88
1	149.11	14.84	144.56	13.71	154.76	15.29	160.07	14.81
2	155.57	15.96	138.88	14.28	142.92	15.41	156.34	16.02
3	153.49	14.81	141.31	14.92	139.50	13.16	154.88	16.86
4	157.14	14.72	149.25	14.40	140.83	13.96	162.13	16.06
5	159.68	16.20	148.04	14.51	139.96	14.39	160.63	16.63
6	153.15	16.20	146.80	13.38	144.49	15.21	158.99	15.74
7	145.27	14.60	148.96	15.65	143.42	14.00	158.11	16.07

Table 5-14. Effects of VAI feeding on Organ Indexes (g/100g, Mean +/- SD)

	Control		Low		Med.		High	
Organs	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Heart	0.33	0.04	0.40	0.04	0.42	0.08	0.32	0.06
Liver	4.34	0.37	4.58	0.42	4.58	0.40	4.51	0.43
Spleen	0.63	0.03	0.59	0.05	0.60	0.05	0.56	0.06
Lung	0.62	0.25	0.92	0.09	1.00	0.10	0.98	0.14
Kidney	0.49	0.08	0.48	0.04	0.49	0.10	0.46	0.13

Table 5-15 Effects of VAI feeding on Heart Rate (per minute, Mean +/- SD)

	Control		Low		Med.		High	
Month	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	374.3	39.9	376.3	40.3	351.2	35.2	347.5	34.5
1	377.9	36.5	368.8	37.3	345.0	36.7	332.2	35.1
2	382.2	38.7	352.1	37.2	323.4	33.3	347.1	34.9
3	356.4	35.6	348.6	35.7	326.5	34.3	343.7	32.0
4	376.0	36.9	354.5	35.2	335.1	34.1	319.4	32.1
5	394.9	39.9	343.1	35.1	338.5	32.6	335.9	32.0
6	374.3	38.9	364.1	33.6	369.1	34.6	341.8	33.2
7	366.5	37.9	365.0	36.3	372.3	35.7	356.5	33.4

5.1.2.3.5 Other evaluations

The list of parameters that were tested in this 30 week oral toxicity study are given above in 5.2.2. No abnormality was found at any dose level of V-411 administration that was significantly different from the control groups in both rats and dogs. This was true for the macroscopic and microscopic histological evaluations of all tissues examined in both species.

5.1.2.3.6 Conclusions chronic toxicity

Chronic oral ingestion of V-41 I (daily for 30 weeks) by dogs and rats at doses that were between 500 and 2000 times the effective dose in rodents was not associated with any organ toxicity when compared to a concomitant, parallel negative control group of treated animals.

5.1.3. CONCLUSIONS ANIMAL TOXICOLOGY & SAFETY

Four animal species were used in this series of studies (mice, rats, digs and rabbits). The findings indicate that V-411 is well absorbed following oral administration and probably has a plasma half life of 10 hours (to be confirmed). It is devoid of harmful reproductive, teratogenic, mutagenic and carcinogenic effects in the animal species tested. The LD 50 was 20 g/kg, and in mice the therapeutic index was greater than 10,000. Daily oral administration over a period of 30 weeks (280 days or 7.5 months) at doses that were 500 to 2000 times the effective dose in rodents and dogs was free of any significant idiosyncratic or dose related toxicity that could be attributed to the drug.

This is a remarkably favorable animal toxicity profile for an anti-diabetic drug.

5.2 MECHANISM OF ACTION

Some studies were carried out in Richmond, Canada as part of the discovery process, and the Chinese studies were carried out at the Academy of Military Medical Sciences and Hebei Medical University. The latter were an integral part of the NDA approved in that country. The mechanism of action evaluation was based on the types of studies listed below.

BIOLOGICAL STUDIES ON RODENT MUSCLE CELLS "in vitro"
- **Insulin Binding Kinetics**
- **Insulin Internalization Kinetics**
- **Glucose & L-leucine Transport**

PHARMACODYNAMIC STUDIES IN DIABETIC RATS "in vivo"
- **Intravenous Drug Administration**
- **Oral Drug Administration**
- **Short and Chronic Drug Administration**
  - **unformulated**
  - **formulated**

5.2.1 Biological Studies on Rodent Muscle Cells (in-vitro)

These investigations were designed to see if V-41 I was modulating metabolism by modifying the behavior of insulin at the cellular level, and/or modifying the transport of glucose or a branched amino acid like L-leucine, across the plasma membrane.

5.2.1.1 Kinetics of insulin binding to muscle cells

It is possible to identify specific insulin receptors on the surface of living cells by binding experiments using [I-125]-labeled insulin (A. Ciechanover, A. Schwartz, and H.F.Lodish, 1983, Cell 32:267-275). The total binding using this method consists of the specific binding of insulin to high-affinity receptors as well as nonspecific low-affinity sticking to other molecules on the cell surface.

Schwartz et al showed that a single hepatoma cell has about 40,000 insulin receptor molecules per cell. The insulin receptor is a ligand-activated protein kinase and is present as I part per 10,000 of cellular protein.

This series of experiments were designed to see whether V-411 altered the insulin binding capacity of rat skeletal muscle cells using [1-125)-labeled insulin as specific receptor probe.

Skeletal muscle cell suspensions were prepared by mechanical disaggregation. A constant number of muscle cells was incubated with a mixture of [I-125)- labeled insulin and one of the six following test substances -buffer (blank control), or Drug 1 and 2, commercially available drugs (These drugs included a sulfonyl urea and a

biguanide.), or Drug 3 and 4 (Mannitolatodimolybdate Complex and Radix Trichosanthis, respectively).

The mixture which was incubated consisted of :

[muscle cells + [1^{-125})-labeled insulin +test substance]

was shaken and then incubated in a refrigerator for I hour at 6 C. The tubes were then placed in a 37C incubator for varying periods of time. A set of tubes was removed at times : 0, 10, 20, 30 and 60 minutes. The tubes were quickly centrifuged and washed with 4'C Krebs-Ringer solution. The remaining radioactivity was read in a gamma counter and expressed as counts per minute per test. The mean and standard deviation were calculated for each test point and the student t-test used to measure statistical significance of the difference between means. The results are expressed in numeric and graphic form below.

5. 2.1.1 Results

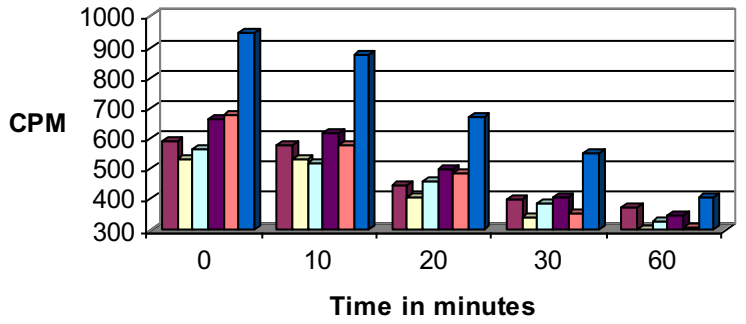
Peak reactions were seen in the "0" time tubes. Hence whatever the nature of the phenomenon it occurred in seconds to a minute after incubation. A list of reactions and their timing is given later (see section 10.0).

The peak cpm counts were 589 and 949 for controls and V-41 I tubes respectively, an increase of 61 %. If one measures the AUC (area under the curves, see appendix 2) the areas were 95.5 and 235 respectively, an increase of 146% in favor of V-41 1. The 50% downregulation in binding occurred between 30 and 60 minutes for V-41 I and > 60 minutes for the control. The differences between control and V-411 counts are highly significant ($p < 0.01$) throughout.

Table 16 Effects of V-411 & other drugs on insulin binding on rat muscle

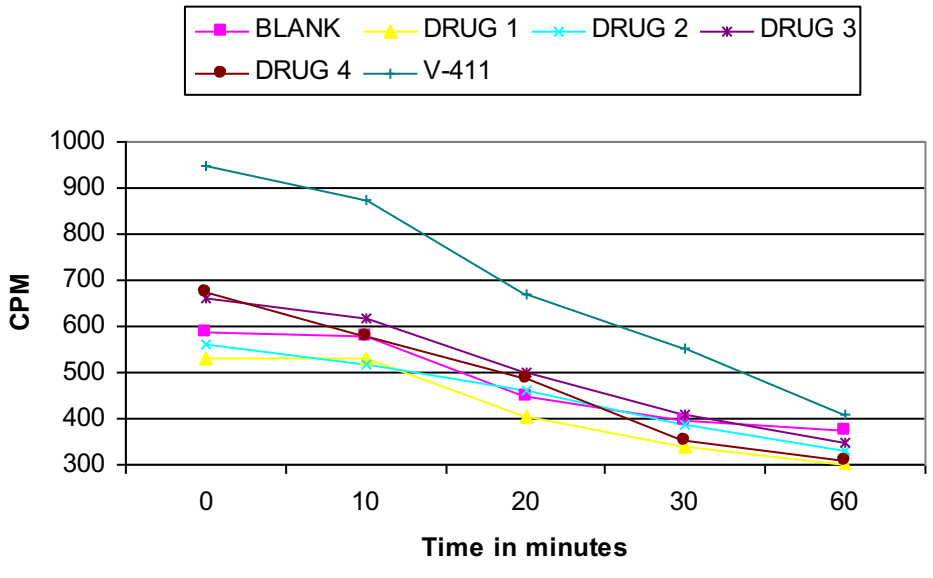
COUNTS PER MINUTE						
	BLANK	DRUG 1	DRUG 2	DRUG 3	DRUG 4	V-411
TIME (min)						
0	589 +/- 39	530 +/- 24	563 +/- 25	663 +/- 29	675 +/- 30	949 +/- 42
10	579 +/- 19	529 +/- 20	517 +/- 19	616 +/- 23	579 +/- 21	876 +/- 33
20	447 +/- 23	405 +/- 21	460 +/- 23	499 +/- 25	487 +/- 25	671 +/- 34
30	396 +/- 17	341 +/- 14	386 +/- 16	408 +/- 17	354 +/- 15	551 +/- 23
60	372 +/- 12	301 +/- 10	329 +/- 11	346 +/- 11	307 +/- 10	407 +/- 13

Figure 2 Effect of V-411 on insulin binding kinetics on rat muscle cells



Hence when rat muscle cells are incubated at 37 C there is an increase in their ability to bind insulin. This ability is dramatically increased when the cells are cultured in the presence of V-41 1. The increase varies between 61 % peak and 146% AUC effects. By 60 minutes the effect is mostly gone, under the conditions of the study.

Figure 3 Insulin binding is more greatly increased following V-411 compared to other diabetic drugs



V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.

The 2 other anti-diabetic drugs had a much less dramatic effect on insulin binding. In fact drugs 1 and 2 responded like, or just below the control buffer specimens.

5.2.1.1 Conclusion insulin muscle receptor binding

Insulin binding of rat muscle cells is rapidly increased at 37 C. This increase lasts from 30 to 60 minutes. The magnitude of this response is considerably augmented by the addition of V-41 1, but minimally by other anti-diabetic drugs. The kinetics however remain relatively constant.

V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.

An explanation for these observations is that insulin receptor expression and downregulation is temperature dependent. Receptor activity (insulin binding), is increased by V-411 either numerically or functionally. This up-regulation boost does not alter the receptor turnover cycle.

5.2.1.2 Kinetics of insulin internalization in rat muscle cells

These experiments were done using radio-labeled insulin, which was iodinated using [I^{125}](by xv.method). Rat skeletal muscle cells, were prepared and the test solutions and incubation times were exactly the same as described in 5. 1.1 except that the "0" time was not included.

The important difference was that after 37 C incubation and washing in cold Krebs solution, the cells were enzymatically disrupted using trypsin, then washed in saline and filtered. It has previously been demonstrated that this technique allows for internalized insulin to be measured, membrane receptor insulin having been removed and discarded by the enzymatic treatment and washings.

5.2.1.2 Results

Over a period of 60 minutes the peak concentration of internalized insulin increases from 45 cpm to a peak of 111 cpm in the control tubes, an increase of the order of 145%. When V-411 is added the peak increase is 177% (181 cpm to 501 cpm). The differences between peak concentrations plus control and V-411 is 35 1 % (111 cpm

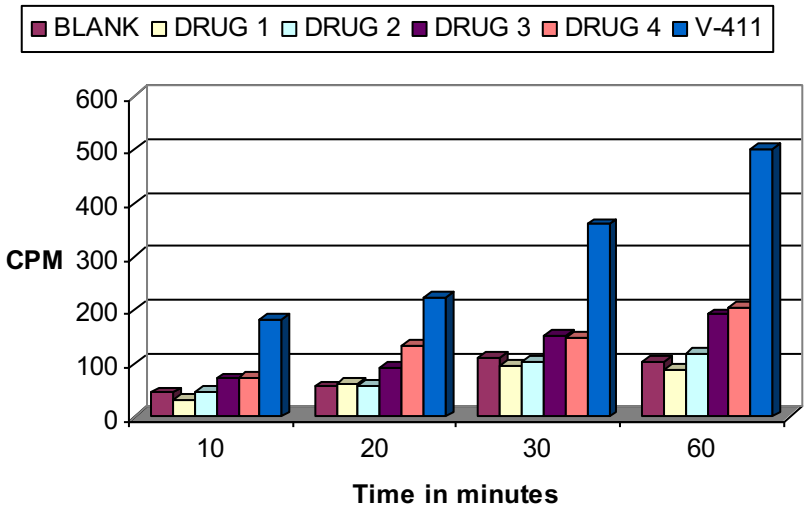
vs. 501 cpm respectively). If the AUC are calculated (see appendix 2) the difference is even more marked, 650% (24 vs. 180 respectively). These differences are highly significant (p value <0.01).

Table 17 Kinetics internalization of insulin in rat muscle cells

COUNTS PER MINUTE						
TIME (min)	BLANK	DRUG 1	DRUG 2	DRUG 3	DRUG 4	V-411
10	45 +/- 2	31 +/- 2	48 +/- 3	71 +/- 3	72 +/- 5	181 +/- 13
20	56 +/- 4	61 +/- 5	57 +/- 4	93 +/- 4	134 +/- 7	224 +/- 12
30	111 +/- 7	96 +/- 4	102 +/- 6	153 +/- 8	148 +/- 9	360 +/- 22
60	104 +/- 9	89 +/- 9	118 +/- 9	191 +/- 12	205 +/- 15	501 +/- 27

In addition the rise in internalized insulin is still ongoing after 60 minutes of incubation with V-41 1, whereas in the buffer control levels begin to fall after 30 minutes. It would have been interesting to see how long V-411 could have continued to cause an increase in insulin endocytosis.

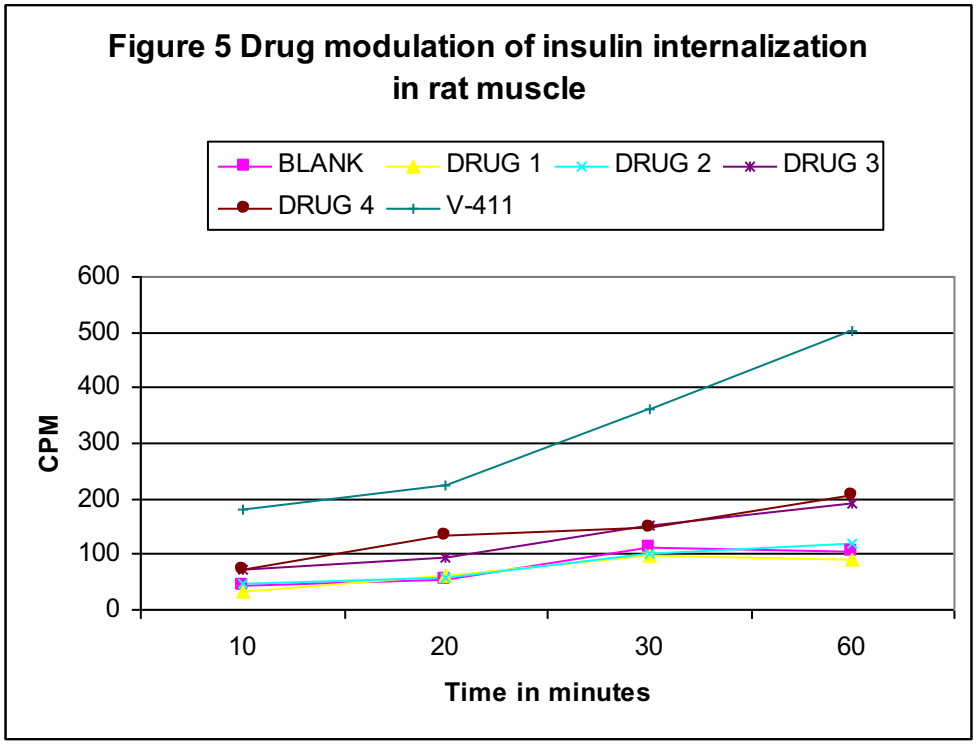
Figure 4 Effect of V-411 on kinetics of internalization of insulin in rat muscle cells



Drugs 3 and 4 cause a significant increase in internalized insulin compared to buffer control, 173% and 185% (increase from 10 min. to 60 min.) respectively. The differences over buffer are highly significant (p value <0.01), but several orders of magnitude lower than the V-41 I effect.

V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of

V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.



5.2.1.2 Conclusions

Following up-regulation of insulin receptors on muscle cells, endocytosis of insulin is triggered; this begins within minutes of receptor activation and peaks around 30 minutes before failing off. Some anti-diabetic drugs cause an increase in this phenomenon, some do not, but V-41 I was the most active of them all.

V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.

5.2.1.3 Modulation of glucose transport by rat muscle cells.

It is possible to measure the intracellular incorporation of glucose by tracking an appropriately labeled preparation. A suspension of rat muscle cells was prepared as 3 in 5. 1. 1. The cells were incubated for I hour at 37 C in the presence of [6-^{3}H]-glucose (50 μCi/mmol), plus or minus insulin, DMEM culture medium and one of the following test compounds: either buffer (blank), or Drugs 1, 2, 3 or 4, or V-41

1. Following incubation the incubated test samples were washed in cold saline and counted in a beta counter.

The mean and standard errors were calculated for each test point, and the Student t test used to determine the statistical significance of differences between the means. The results are presented in tabular and graphic form below.

5.2.1.3 Results

The results show that the transport of glucose into rat muscle cells was by the addition of insulin to cell cultures to a variable degree.

Table 18 Effect of V-411 and other drugs on insulin induced glucose uptake by rat muscle cells

GLUCOSE UPTAKE CPM	BLANK	DRUG 1	DRUG 2	DRUG 3	DRUG 4	V-411
(-) insulin	486 +/- 24	486 +/- 29	582 +/- 26	520 +/- 41	550 +/- 35	532 +/- 28
(+) insulin	682 +/- 57	582 +/- 47	591 +/- 51	1014 +/- 85	1024 +/- 94	1413 +/- 134
% change	40%	20%	2%	95%	86%	166%

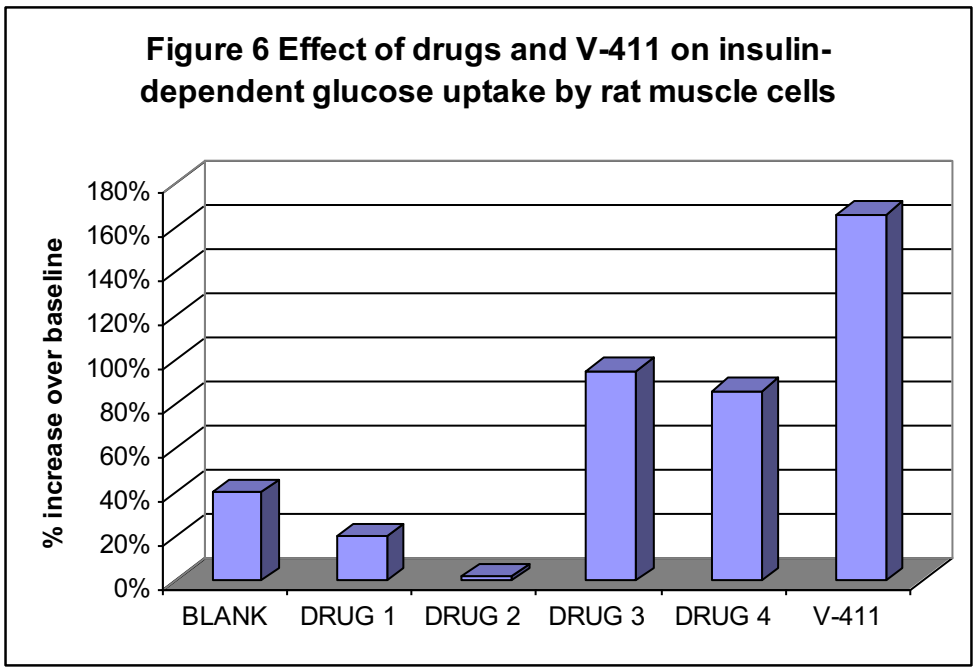
Table 19 Comparison of drug induced glucose uptake increase relative to buffer

TEST COMPOUND	% change vs. Blank	
	(-) INSULIN	(+) INSULIN
DRUG 1	0	-15
DRUG 2	20	-13
DRUG 3	7	49
DRUG 4	13	50
V-411	9	107

In the absence of insulin all test samples showed a significant transport of glucose into rat muscle cells. This was relatively constant, and taking the buffer blank as a reference point, 486 cpm, the variation from this level was always in the positive direction with a maximum reading of 582 cpm, i.e. an increase of 96 cpm, or 20%, which is at the lower end of significance.

There was another type of glucose uptake which was insulin dependent. This type of increase in glucose uptake varied from 2% to 166% above noninsulin dependent levels. The greatest increase was seen with V-411 and the lowest with drug 2. The rank order of effect was: V-41 1, Drug 3, Drug 4, Buffer, Drug 1 and finally Drug 2.

The increase in insulin dependent glucose uptake for the top three (V-411, Drug 3 and 4) were highly significant (p value <0.001). The increase for the buffer control was significant (p value < 0.05). These data are graphically displayed for effect in figure 6 below.
When the glucose response with insulin is compared to the buffer control under the same experimental conditions, some of the anti-diabetic drugs namely Drugs I and 2, had a response that was less than the buffer level (but these were not statistically significant, p value >0.05). The effect of glucose uptake without insulin was on the borderline of significance for Drug 2.



V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.

5.2.1.3 Conclusions

Following incubation for 60 minutes at 370C in culture medium, a significant amount of glucose was able to enter rat muscle cells without insulin. In the presence of insulin this was significantly increased. When buffer alone was present, the insulin-dependent-increase of 40% was significant. This increase was further enhanced by the addition of some, but not all, diabetic drugs. The most active in the series tested was V-41 I with an increase of 166% which was greater by far the most active in the series tested.

V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor

binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.

5.2.1.4 Modulation of L-leucine transport by rat muscle cells

The same experimental conditions were used as described in 5.1.3 with the essential difference that glucose was replaced with [4,5- ^{3}H]-L-Leucine.

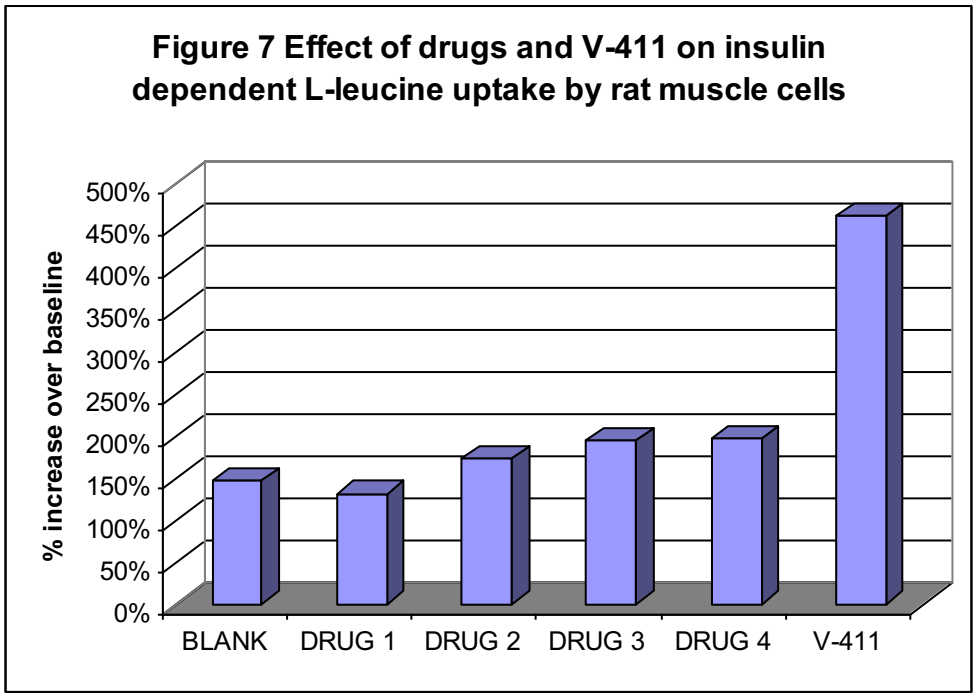
5.2.1.4 Results

L-leucine is taken up by rat muscle cells irrespective of the presence of insulin. The insulin-dependent increase in L-leucine uptake was even more marked than with glucose. The results are tabulated below and graphically displayed for effect has also been produced from the derived data.

Table 12 Effect of V-411 and other drugs on insulin induced L-leucine uptake by rat muscle cells

LEUCINE UPTAKE CPM	BLANK	DRUG 1	DRUG 2	DRUG 3	DRUG 4	V-411
(-) insulin	73.9+/-4	74.1+/-6	70.2+/-6	76.9+/-5	76.3+/-4	77.8+/-5
(+) insulin	180.9+/-11	171.6+/-10	192.5+/-12	227.1+/-17	226.4+/-10	438.8+/-28
% change	147%	132%	174%	195%	197%	463%

Insulin free uptake of L-leucine uptake was identical in all test situations . On the other hand the increase in cell uptake in the presence of insulin varied from a minimum of 132% for Drug I to a maximum of 463% with V-41 1. The descending rank order of degree of uptake difference in relation to insulin was: V-41 1, Drug 4, Drug 3, Drug 2, Buffer, Drug 1. These differences are all highly significant (p values of < 0.01 and <0.001).



V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.

5.2.1.4 Conclusions

L-leucine, under the experimental conditions described was able to enter rat muscle cells when these were incubated in a suitable tissue culture medium at normal physiological temperatures. The amount of L-leucine that moved into the cell was much increased by the addition of insulin either alone or with anti-diabetic drugs. Of the latter V-411 was nearly two fold more active than the others (463%).These were all highly significant increases (p values <0.01 to <0.001)

V-411 is a combination of Mannitolatodimolybdate complex and Radix Trichosanthis. This results shows a clear synergistic effect on insulin receptor binding between Mannitolatodimolybdate Complex and Radix Trichosanthis. All the doses used in this experiment have been optimized. Therefore the effects observed can be considered Maximal Efficacies. This study shows that Maximal Efficacy of V-411 is greater than that of Mannitolatodimolybdate Complex and Radix Trichosanthis.

5.3 ANIMAL PHARMACODYNAMIC STUDIES

These experiments were designed to establish whether the in vitro biological activities in cultured cells could be translated into meaningful pharmacodynamic behavior in an animal model as a prelude to testing in man. In addition it would be possible to test whether the route of administration and formulation affected the response if one was found. A standard rat diabetes model was used.

5.3.1 Effect of intravenous V-41 I on hyperglycemia in diabetic rats.

Diabetes was induced in rats with streptozotocin, which is a nitrosourea (methylnitrosourea) with a special affinity for beta islet cells of Langerhans in the pancreas. The diabetic rats were given sufficient intramuscular injections of insulin to bring down glucose levels by about one third. They were then given a single intravenous injection of a test drug from one of the following: buffer, control Drug], control Drug 2, low dose V-411, medium dose V-411 and high dose V-41 1. Blood samples for glucose measurements were taken at various intervals from 0 to 600 minute (10 hr.). The mean and standard error were calculated for each test/time point and single factor analysis of variance (ANOVA) was used to measure whether the fall in blood glucose was significant

5.3.1 Results

The results show that except for control buffer all test drugs corrected the hyperglycemia to varying degrees. The data are presented in table and graphic form below.

Table 13 Results of pharmacodynamic study in diabetic rats using intravenous V-411 and other drugs

TIME (HR)	BLANK	DRUG 1	DRUG 2	V-411 low dose	V-411 med. dose	V-411 high dose
0	414.6±12.5	414.9±13.6	421.2±10.3	405.0±13.3	422.0±10.1	421.3±13.4
2	401.4±10.6	382.9±12.0	317.7±6.6	337.8±9.5	307.9±6.8	302.1±7.1
4	404.8±12.0	376.5±13.5	303.88.3	306.6±8.6	266.2±8.0	245.5±5.8
6	392.7±11.3	400.6±10.4	330.1±8.9	314.8±10.0	266.2±8.8	214.7±5.2
8	417.1±12.5	411.6±8.7	355.5±10.7	352.7±8.2	334.7±8.3	316.7±8.4
10	403.1±10.5	424.4±9.6	408.4±14.5	400.7±10.7	386.2±12.5	379.7±10.2

Figure 8 Dose response pharmacodynamics after IV V-411

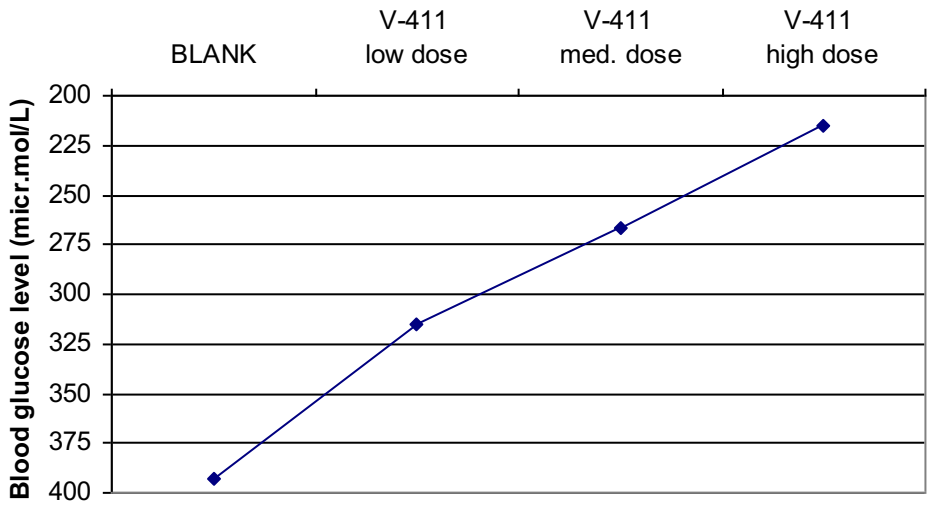
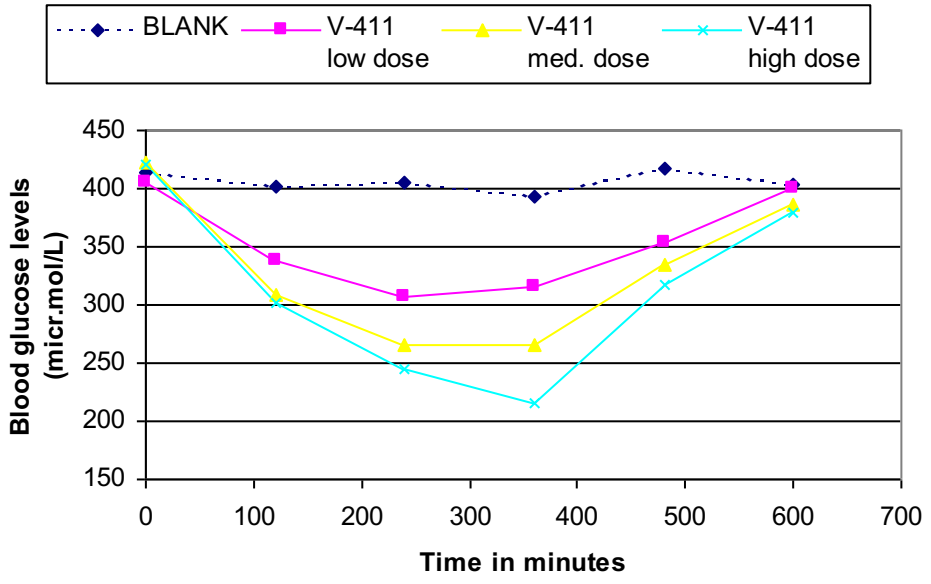


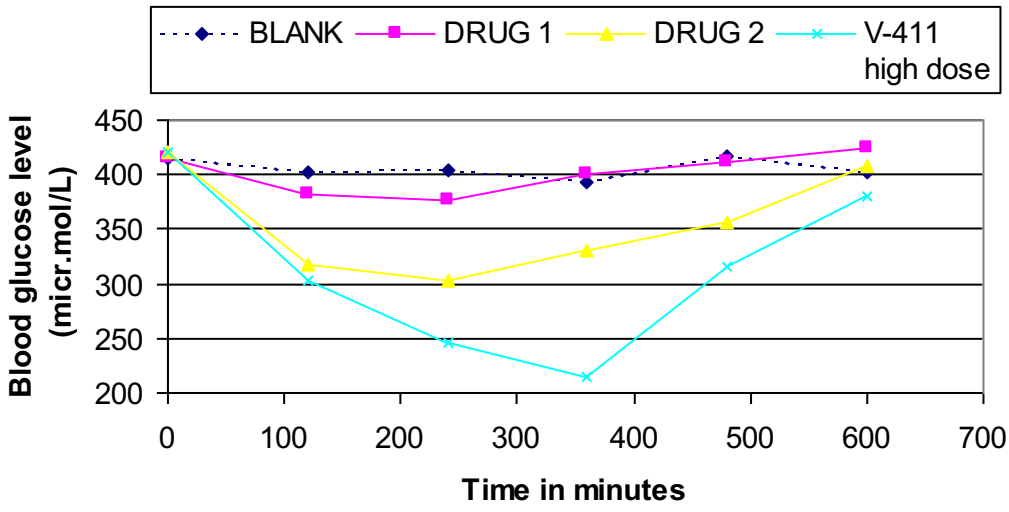
Figure 9 Dose-response hypoglycemic effect of intravenous V-411 in rats



No significant drop in blood glucose was seen in rats treated with intravenous buffer, whereas those treated with all three doses of intravenous V-411 showed very significant (single factor ANOVA $p<0.001$) drop in blood glucose. The peak fall occurred between 4 and 6 hours and had virtually disappeared by 10 hours. There was

clearly a dose response effect. From the data presented it is not possible to know if the "high" dose of V-411 was the maximum effective dose in this model.

Figure 10 Comparative intravenous pharmacodynamic effects of different drugs and V-411



Drug 1 was mildly active, Drug 2 seemed to produce the same degree of hypoglycemia as the low dose of V-411. The high intravenous V-411 dose produced the greatest pharmacodynamic effect.

5.3.1 Conclusions

A single intravenous injection of V-41 I produced significant falls in blood glucose at all dose levels studied. The two comparator drugs were much less effective. The high dose iv V-41 I dose produced the most profound effect. The hypoglycemic effect peaked between 4 and 6 hours and had virtually disappearing by 10 hours.

5.3.2 Pharmacodynamic effects of sub-chronic oral daily administration of V-411 and other drugs in diabetic rats.

The experiment was set up as described in 5.2.1 above, except that the test substances were administered orally. They consisted of placebo control, V-411 (low, medium and high doses) and anti-diabetic Drugs 1 & 2. Test substances were given daily for 48 days. Blood glucose levels were measured every 4 days. The mean and standard errors for each test point/treatment group was calculated.

5.3.2 Results

The results are tabulated below and presented graphically.

Table 14 Results of oral pharmacodynamic study of V-411 and other drugs in diabetic rats

TIME days	PLACEBO	DRUG 1	DRUG 2	V-411 Low dose	V-411 Med dose	V-411 High dose
0	402.2+/-18.2	376.0+/-13.7	401.6+/-17.3	412.9+/-20.1	411.5+/-15.8	388.1+/-12.9
4	409.4+/-17.6	398.4+/-15.6	346.2+/-15.8	326.9+/-10.9	314.4+/-11.0	276.6+/-10.5
8	390.3+/-17.1	383.9+/-17.4	291.6+/-9.2	288.0+/-9.0	217.3+/-7.0	178.4+/-5.6
12	405.4+/-17.1	401.7+/-15.8	281.9+/-11.8	283.8+/-13.3	200.1+/-8.9	202.4+/-7.4
16	401.8+/-14.6	405.1+/-18.2	298.7+/-11.0	289.9+/-11.3	204.7+/-8.8	182.4+/-5.4
20	415.6+/-20.2	424.4+/-15.1	283.8+/-9.5	276.6+/-6.1	190.4+/-6.5	193.1+/-8.9
24	392.5+/-13.9	413.0+/-19.0	275.9+/-12.0	296.6+/-7.9	204.0+/-9.9	176.0+/-6.2
28	391.8+/-16.7	397.0+/-13.0	283.2+/-10.6	303.3+/-10.0	207.9+/-5.5	170.1+/-7.9
32	404.7+/-15.6	404.9+/-17.7	281.6+/-8.3	287.7+/-9.0	191.6+/-6.0	194.9+/-7.1
36	391.7+/-16.1	411.2+/-14.9	292.4+/-12.0	277.3+/-10.6	220.9+/-8.3	193.6+/-8.6
40	398.1+/-17.1	391.0+/-20.2	280.1+/-13.0	300.8+/-10.0	216.3+/-10.1	185.7+/-8.0
44	411.6+/-17.7	384.3+/-15.8	273.8+/-11.1	276.8+/-11.5	206.2+/-7.9	183.3+/-6.1
48	405.7+/-17.5	410.8+/-18.0	278.9+/-11.4	286.9+/-10.0	219.4+/-5.2	189.4+/-6.5

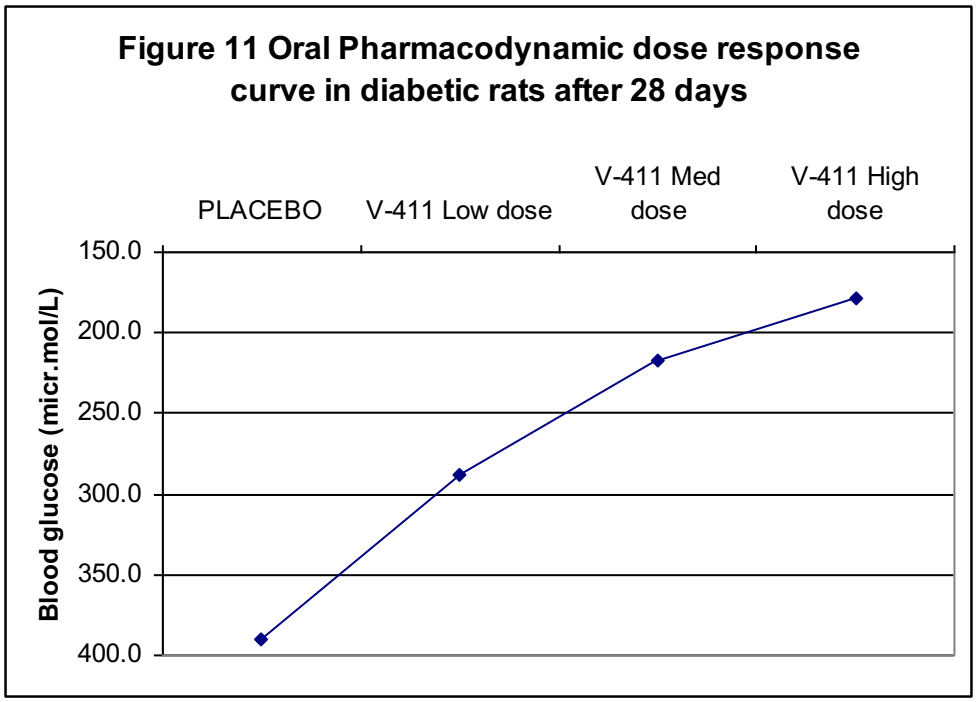
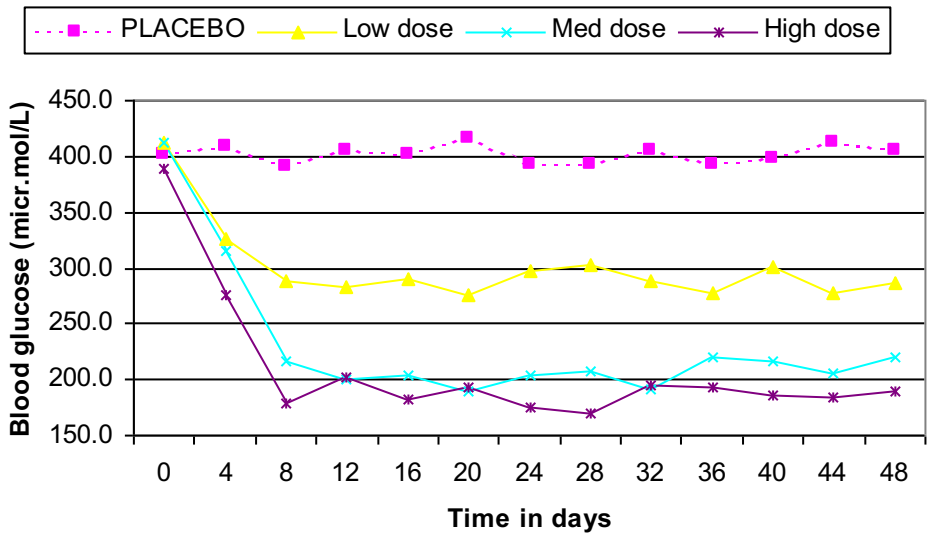


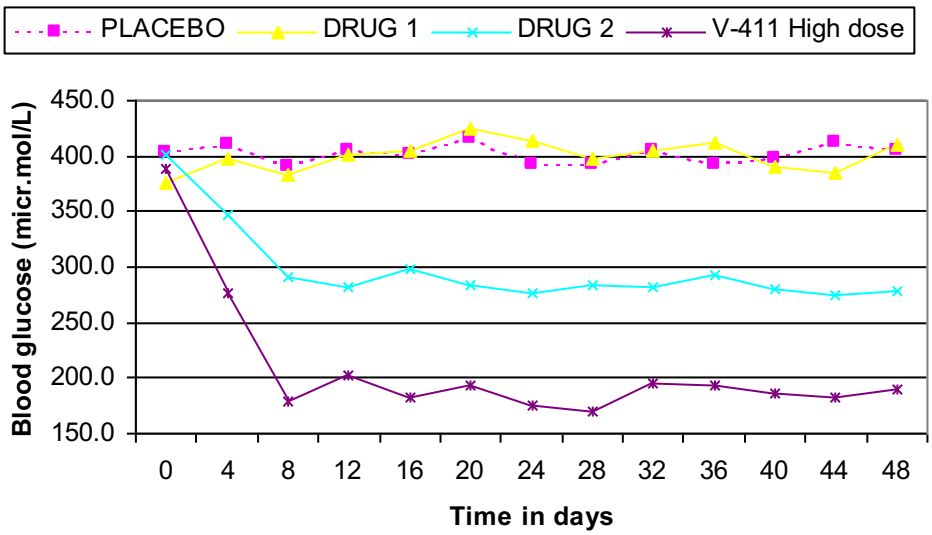
Figure 12 Dose response pharmacodynamic effects of oral V-411 in diabetic rats



The data show oral V-411 was efficient in lowering blood glucose in diabetic rats. All dose levels were effective and the medium and high dose levels effects were close suggesting that the optimal dose in this model was virtually achieved with the high dose. This was most clear at 48 days when the dose effects had stabilized. The effect took 8 days to optimize and remained constant throughout the 48 days of the study. No response was see with placebo. The error dispersion around the mean were ± 5% or less. These decreases in the mean blood glucose was highly significant using the single factor ANOVA analysis, p value <0.001.

One of the two other drugs tested (Drug 2) was effective as the low dose oral V-41 1. They other was indistinguishable from placebo.

Figure 13 Comparison pharmacodynamic effects of oral V-411 and hypoglycemic drugs in rats



The maximum fall in blood glucose level following multiple high dose oral V-411 was 170.1 ± 7.9 micromol/L, which was a 56% decrease from time zero values, and following a single high dose of intravenous V-411 was 214.7 + 5.2 micromol/L, which was a 50% decrease from base line values.

5.3.2 Conclusions

Diabetic rats responded well to all doses of daily oral V-41 I at the three doses examined. The effect was near optimal by day 8 of therapy and was maintained throughout the 48 day treatment period. Only one of the comparator drugs was effective, and the level of response was close to the low V-411 dose response. There was a dose pharmacodynamic response in the diabetic rat model.

In terms of optimal response this was best achieved following multiple daily oral high dose treatments of V-411 (56% drop from baseline) compared to the single intravenous dose effect which was 50% drop from baseline.

These data demonstrated that oral V-41 I was highly effective in normalizing the hyperglycemia of experimentally induced diabetes in rats. It's pharmacodynamic profile rendered it a very suitable candidate for human evaluation.

5.4. CLINICAL EXPERIENCE

At this time over 99% of the clinical experience of this drug is derived from the NDA program that was run and coordinated in China. Approval for commercialization was obtained in 1996. Since December 1996 the drug has been marketed in some regions in China and already ten's of thousands of patients have been prescribed the drug.

5.4.1. Pivotal phase III clinical study

5.4.1.1. Study objectives

The primary objective of this study was to test whether V-411 was a safe and effective treatment for diabetes by measuring and comparing objective and subjective criteria of the disease, with those of patients receiving placebo.
The secondary objective of the study was to compare the safety and efficacy of V-411 with a commonly prescribed oral hypoglycemic agent: Glibencamide.

5.4.1.2 Patient Selection criteria

Inclusion criteria:

(1) Patients with diabetic symptoms and fasting glucose level higher than 7.8 mmol/L (140 mg/dL); or
(2) Patients with diabetic symptoms and fasting glucose level lower than 7.8 mmol/L, but 75 gram oral glucose tolerance test (75 g OGTT) results higher than 11.1 mmol/L (200 mg/dL) at 2 hour; or
(3) Patients without diabetic symptoms, but fasting glucose level is higher than 7.8 mmol/L for 2 consecutive times, or 75 g OGTT is higher than 11.1 mmol/L at 2 hour for 2 consecutive times.

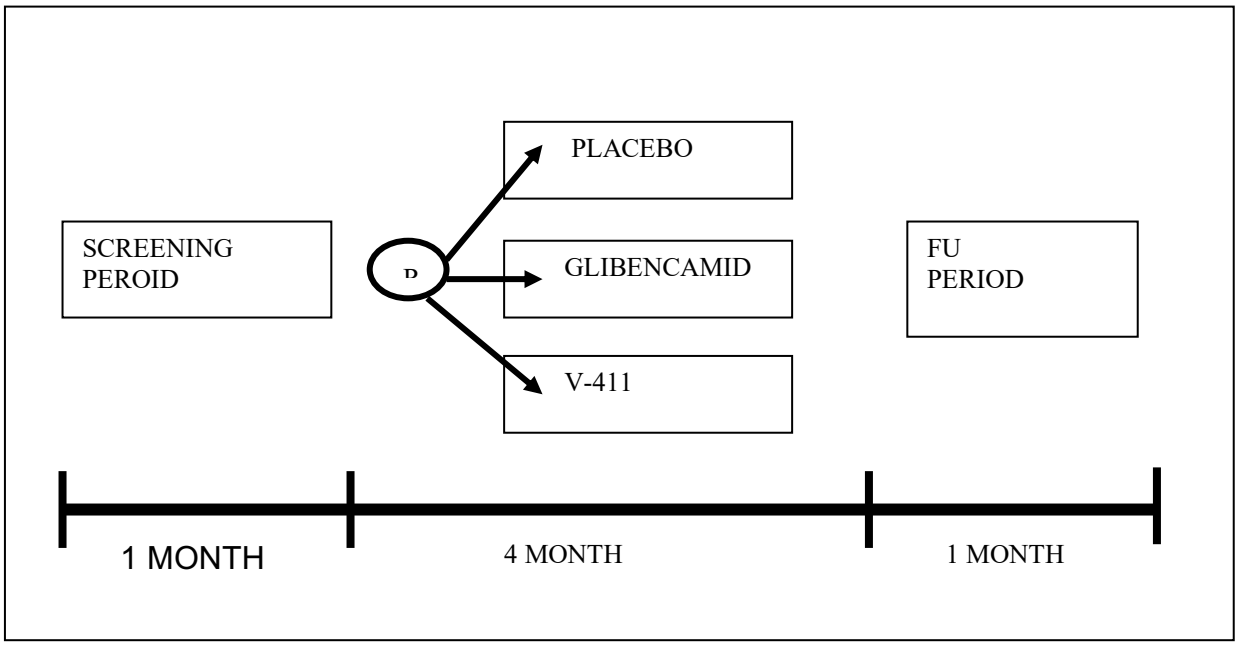
Exclusion criteria:

(1) Patients who do not understand the meaning of this clinical trial, or do not wish to volunteer, or cannot follow instructions from physicians, or
(2) Patients who suffer diabetic ketoacidosis or infection in recent month, or
(3) Patients who are pregnant or younger than 18 years of age, or
(4) Patients with concurrent serious disease or therapy that could interfere with the effects of V-411 or it's evaluation, or
(5) Patients who are receiving concurrent diabetic therapy in the recent month.

1,171 patients and 329 healthy adults were screened for admission. 370 were admitted into the study and were included in the analysis.

5.4.1.3 Study design

This was a double-blind, randomized, parallel triple arm (V-411 versus Glibencamide versus placebo) controlled multi-center phase III study designed to establish the efficacy and safety of orally administered V-41 I in patients with Type II diabetes mellitus or NIDDM (non-insulin dependent diabetes m.)



5.4.1.4 Logistics

Three centers were involved in the study and there was an overall study coordinator. A protocol and a case record form were used to collect the data which were centrally reviewed and controlled for quality. There was a randomization code for the blinded part of the study. Patients were screened and if acceptable randomized to one of three treatment groups.

5.4.1.5 Treatment

Blinded tablets of placebo, V-411 and Glibencamide were prepared by Volque Pharmaceuticals.

Treatment was twice daily for 4 months, one tablet in the morning, one in the evening.

Doses of	V-411	3.2 mg twice daily
	Glibencamide	3.5 mg twice daily
	Placebo	Twice daily

5.4.1.6. Study Parameters
The various study parameters taken at various intervals are listed in the table below

TEST	FREQUENCY			
	ST	Bi-W	D	EN
CLINICAL				
Complete history				
Family history diabetes				
Clinical appraisal				
Complete physical examination				
GENERAL BLOOD TEST				
Renal function tests				
Hepatic function tests				
DIABETES RELATED				
Fasting blood glucose				
Post-prandial blood glucose				
24 hr urine sugar				
ORAL THERAPY				

5.4.1.7. Ethics
The study was approved by Ministry of Health. Informed consent was obtained from all participants.

5.4.1.8. Data and Analyses
Completed CRFs were sent blinded to the study coordinator who managed the database and broke the code when the case was completed. The data from all centers were pooled. The mean and standard deviations were calculated for each parameter. The Student t test was used to test if the difference between groups had achieved statistical significance.

Data were also analyzed using Chi square method, base on the following criteria:
(a) "Very effective": 2/3 of the diabetic symptoms disappeared, and fasting glucose is lower than 7.2 mmol/L, and post-prandial 2 hr glucose is lower than 8.3 mmol/L, and 24 hr urine glucose is lower than 10 g; or the above parameters were reduced by more than 30%.
(b) "Effective": 1/3 - 2/3 of the diabetic symptoms were improved, and the above listed parameters were reduced by 10-29%.
(c) "Not effective": The above listed parameters were reduced by less than 10%.

5.4.2. Results

5.4.2.1. On Glucose
The patients on placebo showed no significant improvement in any of the parameters, when their baseline results were compared with those at the end of treatment. However the two active treatment arms of the study showed highly significant improvement in all three parameters ($p<0.01$)

Table 16 Results of effects on glucose metabolism of clinical trial with 370 patients with type II diabetes mellitus.

PLACEBO	Before	After	"t"	p
Fasting blood glucose (mmol/L)	11.67±2.35	11.10±5.54	0.62	>0.05
2-hr Glucose Tolerance Test (mmol/L)	17.67±6.01	15.99±5.27	2.29	>0.05
24 hr Urine glucose (g/24 hr)	26.66±10.6	25.87±12.5	0.96	>0.05
GLIBENCAMIDE				
Fasting blood glucose (mmol/L)	11.27±2.18	9.79±1.65	4.54	<0.01
2-hr Glucose Tolerance Test (mmol/L)	17.13±3.20	14.27±2.22	6.18	<0.01
24 hr Urine glucose (g/24 hr)	27.30±6.85	23.59±6.09	6.70	<0.01
V-411				
Fasting blood glucose (mmol/L)	12.55±3.13	9.60±2.38	9.23	<0.01
2-hr Glucose Tolerance Test (mmol/L)	17.99±5.2	13.68±4.42	8.74	<0.01
24 hr Urine glucose (g/24 hr)	21.55±11.5	13.30±8.95	8.78	<0.01

When the results are expressed as a percent improvement in each parameter as are shown in table 2 and figure 1, the striking difference in objective improved efficacy between the three treatment arms is readily seen. The improvement produced by Glibencamide treatment is highly significant when compared to placebo. The improvements produced by oral V-411 therapy are significantly better than both placebo and Glibencamide.

Table 17 Glucose results expressed as percent improvement for a specific parameter from the beginning to end of treatment.

Parameters	Placebo	Glibencamide	V-411
Fasting Blood Glucose	4.5%	13.1%	23.5%
Glucose Tolerance Test	9.5%	16.7%	24.0%
Daily Urine Glucose	3.0%	13.6%	38.3%

Fig14 Graph of improvements in glucose metabolism during study displayed by treatment group

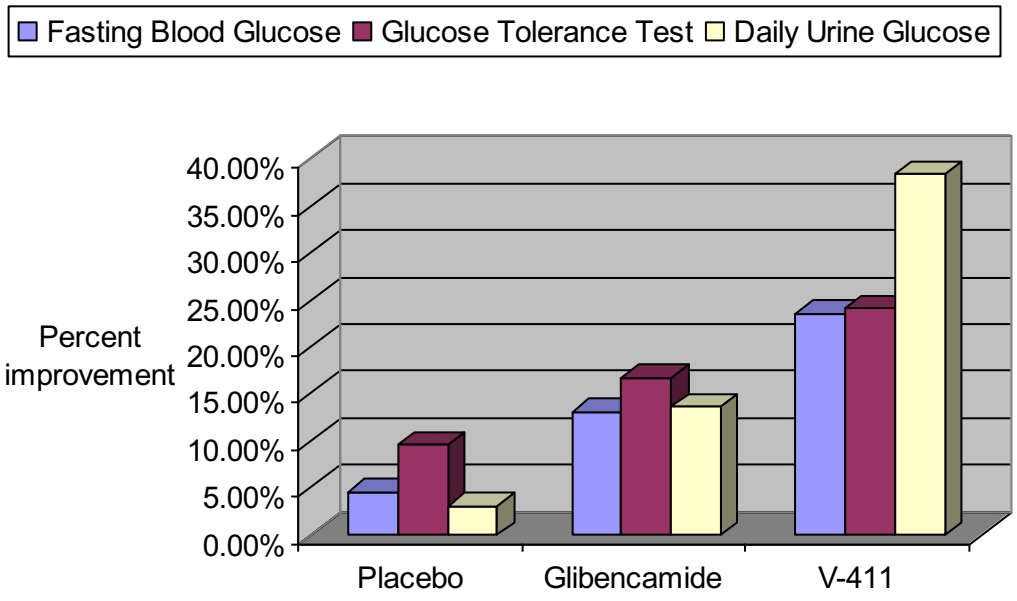
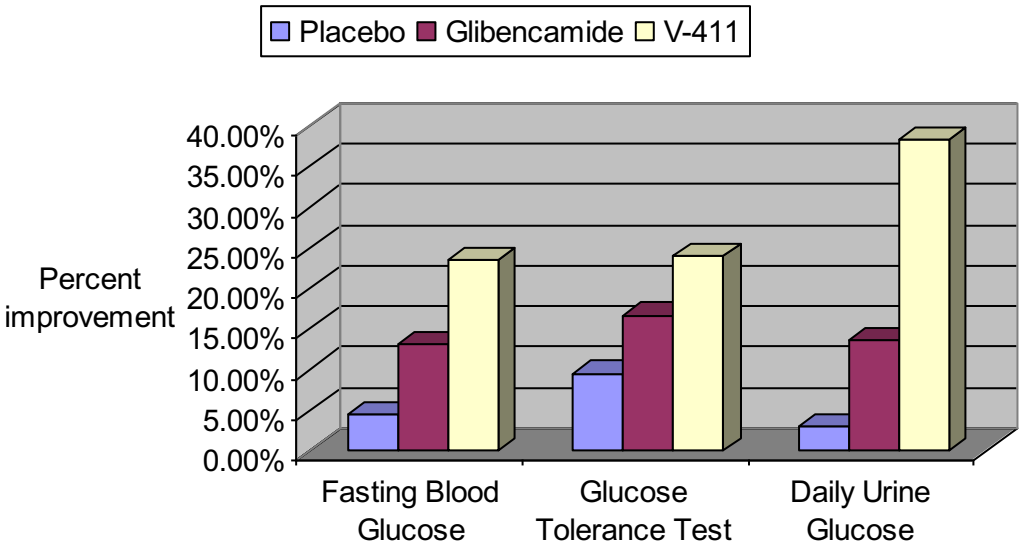


Figure 15 Graph of effects of treatment displayed by parameter tested



The data from the trial were also analyzed for "effective rate". The analysis is tabulated in the following table.

Table 18 Comparison of effective rate between V-411 and placebo and controls. Note: X^2 is calculated based on: "Very Effective"+"Effective", V-411 group vs. Placebo.

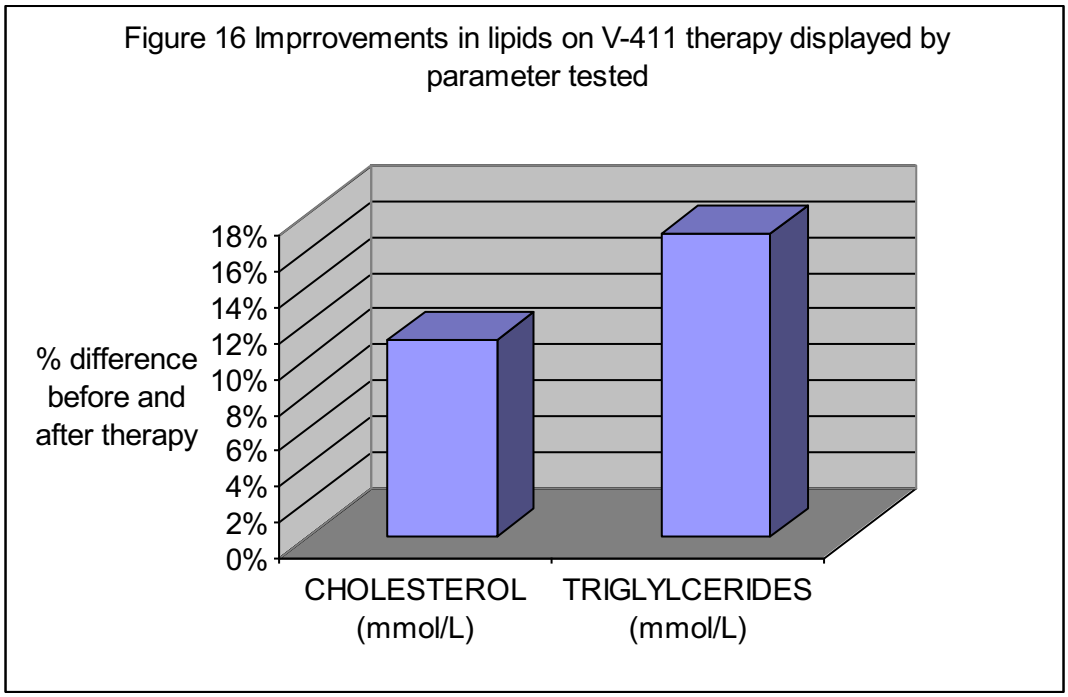
	Very Effective	Effective	Not Effective
Placebo	0%	1%	99%
Glibencamide	13%	47%	40%
V-411	50%	37%	13%
X^2		10.83	
p		<0.01	

5.4.2.2. Effects On Lipids

In addition to effects on glucose metabolism, oral V-411 had a significant effect on cholesterol and triglycerides. Relative to base line values there was a decrease of nearly 11% in fasting cholesterol and 17% in fasting triglycerides.

Table 18 Improvements in lipid measurements on V-411 therapy

FASTING LIPID	**Before**	**After**	**"t"**	**"p"**	**% diff**
CHOLESTEROL (mmol/L)	7.11±1.33	6.34±1.00	3.46	<0.01	11%
TRIGLYLCERIDES (mmol/L)	2.34±1.07	1.94±4.73	4.73	<0.01	17%



5.4.2.3. Effects On Symptoms

V-411 therapy was associated with either a loss or reduction in the symptoms of diabetes, which included: polyuria, polydypsia, polyphagia, and fatigue.

The effect of reducing the signs and symptoms of their disease was to produce a very favorable overall response to therapy.

5.4.2.4. Adverse effects
The incidence and spectrum of adverse effects on V-411 therapy could not readily be distinguished from those encountered on placebo.

5.4.2.5. Contraindication
Diabetic ketoacidosis

5.4.3. Conclusion Phase III Study
Adult patients with Type II diabetes who took oral V-411tablets twice daily experienced a highly significant improvement in the glucose and lipid parameters of disease, in addition to a reduction in the clinical signs and symptoms of the disease. Not only was V-411 superior to placebo, it also was significantly better than Glibencamide. There was an overall response rate of 87% and a toxicity profile that resembled placebo.