TOXICITY STUDY ON VICCILLIN S (AN AMPICILLIN-CLOXACILLIN COMBINATION)

——Subacute Toxicity of Viccillin S in Female Beagle Dogs——

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(Received January 17, 1978)

Viccillin S, an antibiotic mixture consisting of equal amouts of sodium ampicillin and sodium cloxacillin, is active against gram-positive and gram-negative bacteria[1].

This paper describes the results of a subacute toxicity study when Viccillin S was administered intravenously to Beagle dogs for 30 days.

Materials and Methods

Drug

Viccillin S consists of sodium ampicillin (468 mg (potency)/g) and sodium cloxacillin (456 mg (potency)/g), totalling 924 mg (potency)/g.

Animals

Fifteen pure-bred female Beagle dogs, weighing 8800 to 14000 g, aged 26 to 37 weeks, were used.

The dogs were fed throughout with a complete dry diet (Spratt's Dog Diet). Each dog was offered 400 g in the morning.

Whenever new food was offered the residue of the previous meal was removed and weighed. In addition, supplements of milk (approximately 200 ml) were offered to each dog on weekdays only throughout the experiment. Water was freely available in the kennels at all times.

Dosing and dosage groups

Viccillin S was freshly prepared for injection each day as a 10% (potency) solution in isotonic saline (0.9% sodium chloride solution). The drug was administered to each animal intravenously *via* the cephalic vein once a day, seven days a week, for a period of 30 days. Initially, the rate of injection was 4 ml/minute, but in the absence of any severe adverse reactions this was increased to approximately 20 ml/minute after the first few days of the study. The dosage levels were calculated assuming a potency of 920 mg/g (actual potency 924 mg/g) and the dosages throughout this paper are in terms of potency.

The 15 dogs were assigned to 5 groups and dosed as follows :

1 Control-isotonic saline (4 ml/kg/day)
2 50 mg/kg/day (0.5 ml/kg/day)
3 100 mg/kg/day (1 ml/kg/day)
4 200 mg/kg/day (2 ml/kg/day)
5 400 mg/kg/day (4 ml/kg/day)

Clinical observations

Clinical signs were recorded daily. Bodyweight was recorded twice a week. Food consumption was recorded daily. Water consumption was recorded throughout the experiment. Ophthalmoscopic examinations were performed once before dosing commenced and then after dosing for 4 weeks. Electrocardiograms (ECG's) were recorded after dosing for 4 weeks. Recordings were made before dosing, immediately after dosing, 30 minutes after dosing and (for the dogs receiving 400 mg/kg/day only) one hour after dosing.

Haematology, biochemistry and urinalysis

Complete sets of laboratory investigations were performed on all dogs once before dosing commenced and again after 4 weeks dosing. The range of investigations carried out is shown in Table 1. Samples of blood were obtained prior to dosing on the day of the examination, the animals being in a fasting condition.

When urinalysis was performed, the supply of water was withdrawn from the animals at mid-day and the urine specimens were collected between 5 p. m. and 9 a. m. the next morning.

Macroscopic and Microscopic examination of the tissues

Oo completion of the dosing period each animal received an intravenous injection of sodium pentobarbitone and was rapidly exsanguinated by inci-

Table 1 Items investigated and units on haematology, biochemistry and urinalysis

Investigation	Units
Haematology	
Erythrocyte sedimentation rate (ESR)	mm/1 hour
Packed cell volume (PCV)	%
Haemoglobin (Hb)	g%
Red cell count (RBC)	mill/mm^3
Reticulocyte count (Retics)	% red cells
Mean corpuscular haemoglobin cocentration (MCHC) = Hb × 100 ÷ PCV	%
Mean cell volume (MCV) = PCV × 10 ÷ RBC	cubic microns
Total white cell count (WBC)	1000/mm^3
Neutrophils (N)	1000/mm^3
Lymphocytes (L)	〃
Eosinophils (E)	〃
Basophils (B)	〃
Monocytes (M)	〃
Platelet count	1000/mm^3
Prothrombin index (PTI)	% of control PT
Biochemistry	
Plasma urea	mg%
Plasma glucose	mg%
Total serum proteins	g%
Albumin	〃
α^1 globulin	〃
α^2 globulin	〃
β globulin	〃
γ globulin	〃
A/G ratio	
Serum alkaline phosphatase (SAP)	KA units
Serum glutamic-pyruvic transaminase (SGPT)	mU/ml
Serum bilirubin	mg%
Sodium (Na^+)	mEq/L
Potassium (K^+)	mEq/L
Urinalysis	
pH	
Volume	ml
Specific gravity (SG)	
Protein	mg%
Total reducing substances	
Glucose	
Ketones	
Bile pigments	
Urobilinogen	
Haemoglobin	

sion of the carotid blood vessels.

Following macroscopic examination of the organs, the principal tissues were removed and weighed. In order to perform the microscopic examination, small portions of the tissues were fixed with 10% buffered formalin and stained.

Results

Mortalities

There were no deaths.

Clinical signs

All dogs receiving 400 mg/kg/day occasionally vomited during or shortly after dose administration.

All dogs receiving 400 mg/kg/day, one dog receiving 200 mg/kg/day, 3 dogs receiving 100 mg/kg/day, 2 dogs receiving 50 mg/kg/day and 3 control dogs occasionally showed body tremors during and immediately after injection.

Most dogs in all groups receiving the test material showed slight relaxation of the nictitating membranes. This was seen on most days during the dosing period in dogs receiving 400 mg/kg/day and was not observed in control dogs.

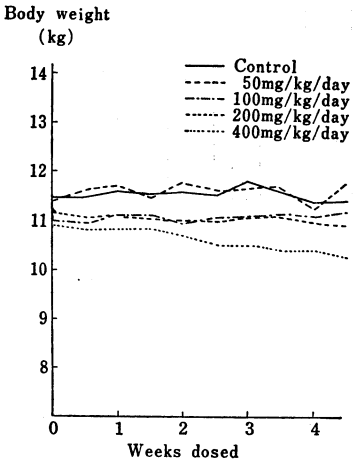
Vasodilation of the peripheral vessels occurred in all dogs receiving 400 or 200 mg/kg/day during or immediately after dosing on most days during the dosing period. This was evidenced by an increase in the skin pinkness where hair cover was sparse on the ventral abdominal surface and the inner surface of the ear pinnae and also by increased pinkness of the mucous membranes of the eyes and mouth. It was noted that the vasodilation tended to be accompanied by an increase in the rate and strength of the heart beat. The increased rate and strength of the heart beat was similar to that which occurred after moderate exercise and tended to persist as long as the vasodilation.

On isolated occasions during the dosing period soft oedematous swellings occurred at the cephalic vein injection sites of two dogs receiving 400 mg/kg/day, all dogs receiving 200 mg/kg/day and 2 dogs receiving 100 mg/kg/day. These tended to persist for up to 3 days.

Bodyweight

Before dosing commenced most dogs gained or maintained weight satisfactorily. Despite minor fluctuations, all 3 dogs receiving 400 mg/kg/day, 2 dogs receiving 200 mg/kg/day, one dog receiving 100 mg/kg/day and one control dog lost or failed to gain weight during the dosing period. Inspection of the group mean bodyweights (Figure 1) showed that the rate of bodyweight gain was similar in the groups receiving 50 or 100 mg/kg/day and the control group. The groups receiving 200 or 400 mg/kg/day showed a mean overall bodyweight loss which was greater in the group receiving 400 mg/kg/day.

Fig. 1 Group mean body weights



Food consumption

During the dosing period all dogs tended to leave greater quantities of food than during the predosing period. In addition, the dogs in the groups receiving the test material tended to leave greater quantities of food than the controls during the dosing period. However, during the predosing period a similar tendency wa also seen.

Water consumption

During the predosing period water consumption was satisfactory for all dogs. During the dosing period most dogs including the controls exhibited a reduction in water intake when compared with

Table 2 Mean heart rate after 4 weeks dosing

mean ± S. D.

Dosage (mg/kg/day)	Before dosing	Immediately after dosing	30 min. after dosing	1 hr. after dosing
control	129±20.7	136±9.7	156±41.4	—
50	132±15.0	136±31.5	157±59.2	—
100	127±23.6	138±20.8	143±32.5	—
200	118±13.0	144*±6.1	135±36.1	—
400	105±11.1	172**±17.6	156*±29.5	136±31.9

* significantly different from the value before dosing in each group $P<0.05$
** significantly different from the value before dosing in each group $P<0.01$

Table 3 Haematological investigations (mean±S. D.)

	Dosage (mg/kg/day)	ESR (mm/hr)	PCV (%)	Hb (g%)	RBC (10^6/mm^3)	Retics (%)	MCHC (%)	MCV (cμ)	WBC (10^3/mm^3)						Platelets (10^3/mm^3)	PTI (%)
									Total	N	L	E	B	M		
Before dosing commenced	control	0	58±4.2	16.6±0.60	6.7±0.59	<2.0	29±1.2	87±1.2	11.3±1.80	6.0	4.5	0.6	0.0	0.1	228±33.3	100±2.1
	50	0	53±1.5	14.8±1.39	6.0±0.45	<2.0	28±2.1	89±3.2	12.0±2.05	6.6	5.4	0.1	0.0	0.0	272±12.6	102±9.3
	100	0	56±2.0	16.7±0.12	6.3±0.12	<2.0	30±1.0	88±2.3	14.1±1.55	7.1	6.5	0.5	0.0	0.0	263±28.4	101±3.5
	200	0	53±2.1	16.0±0.15	6.2±0.35	<2.0	30±1.0	86±7.9	12.7±1.01	6.8	5.6	0.3	0.0	0.0	283±27.5	101±6.4
	400	0	55±1.0	16.1±0.50	6.5±0.12	<2.0	29±0.6	85±1.0	14.3±2.00	6.5	6.8	1.0	0.0	0.2	328±14.4	96±3.1
After dosing for 4 weeks	control	0	52±4.0	17.3±1.54	6.8±0.64	<2.0	33±0.6	77±2.7	9.9±0.90	4.6	4.6	0.7	0.0	0.0	220±30.4	100±4.6
	50	0	52±1.0	16.9±0.52	6.9±0.46	<2.0	32±0.6	76±6.0	10.7±1.68	5.0	5.2	0.5	0.0	0.0	283±37.9	101±9.2
	100	0	51±2.0	16.3±0.76	6.2±0.32	<2.0	32±0	83±5.6	11.3±1.67	5.9	4.5	0.8	0.0	0.2	262±44.8	103±0
	200	0	52±2.6	16.5±0.81	6.0±0.65	<2.0	32±0.6	87±4.6	9.8±1.06	4.9	3.8	0.7	0.0	0.3	290±39.7	103±4.5
	400	0	52±2.5	16.9±1.51	6.5±0.2	<2.0	32±1.5	80±1.2	9.7±2.84	5.3	3.6	0.6	0.0	0.1	305±32.8	102±6.6

Table 4 Biochemical investigations (mean±S. D.)

	Dosage (mg/kg/day)	Urea (mg%)	Glucose (mg%)	Serum proteins (g%)						A/G ratio	SAP (KA units)	SGPT (mU/ml)	Bilirubin (mg%)	Na (mEq/L)	K (mEq/L)
				Total	Alb	α1	α2	β	γ						
Before dosing commenced	control	30±3.5	100±3.6	5.2±0.10	3.0	0.3	0.5	1.1	0.3	1.38±0.098	14±4.0	30±7.6	0.1±0	147±0.6	4.6±0.55
	50	28±2.7	98±6.5s	5.4±0.12	2.9	0.3	0.6	1.2	0.4	1.15±0.110	13±1.2	25±2.1	0.2±0.12	147±0.6	4.8±0.38
	100	29±3.8	96±1.7	5.4±0.31	2.9	0.3	0.5	1.3	0.4	1.22±0.220	11±0.6	25±3.6	0.1±0	147±0.6	4.7±0.69
	200	29±11.6	97±8.1	5.5±0.26	2.9	0.3	0.5	1.3	0.4	1.17±0.190	13±2.9	26±4.5	0.1±0	148±0.6	4.4±0.25
	400	26±5.3	102±6.6	5.2±0.21	2.7	0.3	0.5	1.3	0.4	1.08±0.045	12±2.7	31±15.1	0.1±0	149±0	4.4±0.17
After dosing for 4 weeks	control	32±7.8	103±4.5	5.3±0.15	2.9	0.3	0.5	1.2	0.4	1.23±0.150	15±3.6	34±7.8	0.1±0.06	145±1.2	4.5±0.21
	50	29±4.4	100±4.0	5.5±0.15	3.0	0.3	0.5	1.3	0.4	1.19±0.099	17±3.1	29±3.6	0.2±0.12	146±0.5	4.5±0.10
	100	26±4.2	91±9.5	5.1±0.40	2.9	0.3	0.4	1.2	0.3	1.35±0.133	15±1.2	27±6.6	0.1±0	145±0.5	4.3±0.21
	200	25±3.8	96±0.6	5.3±0.20	3.1	0.3	0.4	1.2	0.4	1.40±0.064	16±2.5	32±3.5	0.1±0.06	146±0.5	4.3±0
	400	27±8.7	100±1.5	5.6±0.12	3.2	0.3	0.4	1.3	0.4	1.37±0.119	20±6.1	40±26.5	0.2±0.06	150±3.2	4.1±0.20

the values obtained during the predosing period.

Ophthalmoscopy

The eyes of all dogs were examined once before dosing commenced and again after dosing for 4 weeks. No abnormalities were detected that could be related to treatment with the test material.

Electrocardiography

Before dosing, heart rates (calculated from R-R interval values, Table 2) were within normal limits.

Immediately after dosing, there was a slight increase in the mean heart rate of the group receiving 200 mg/kg/day and a marked increase in the mean heart rate of the group receiving 400 mg/kg/day.

Thirty minutes after dosing the mean heart rates of the groups receiving 200 or 400 mg/kg/day were less than those recorded immediately after dosing, but were still greater than the predosing values. The mean heart rates of the groups receiving 50 or 100 mg/kg/day and the control group had increased slightly.

One hour after dosing (for dogs receiving 400 mg/kg/day only), the group mean heart rate was slightly above the predose value, although was lower than the values obtained immediately after dosing and 30 minutes after dosing.

All other numerical data (PR, QRS and QT intervals) were within normal limits and no abnormalities of the electrical complexes were seen before or after dosing.

Haematology, biochemistry and urinalysis

The results are presented in Table 3~5.

(i) Haematology

Before dosing commenced, all results were within normal limits.

After 4 weeks dosing, the white bell count of one dog receiving 400 mg/kg/day was slightly below our lower limit of 7,000/mm^3. All other results were within nrmal limits.

(ii) Biochemistry

Before dosing commenced all results were within normal limits.

After 4 weeks dosing the SGPT value for one dog receiving 400 mg/kg/day was above our normally accepted upper limit of 50 mU/ml. This finding was confirmed when a further blood sample was examined on the following day. All other results were within normal limits.

Table 5 Urinalysis

(mean ± S. D.)

	Dosage (mg/kg /day	pH	Volume	Specific gravity	Protein (mg%)	Total red. subs.	Glucose	Ketones	Bile pigment	Urobilinogen	Haemoglobin
Before dosing commenced	control	6.6 ±0.44	106 ±70.8	1.049 ±0.0066	0	—	(−)	(−)	(−)	(−)	(−)
	50	6.7 ±0.36	123 ±15.3	1.041 ±0.0050	0	—	(−)	(−)	(−)	(−)	(−)
	100	6.7 ±0.42	120 ±34.6	1.039 ±0.0051	0	—	(−)	(−)	(−)	(−)	(−)
	200	6.6 ±0.44	77 ± 5.8	1.045 ±0.0044	0	—	(−)	(−)	(−)	(−)	(−)
	400	6.8 ±0.06	129 ±59.9	1.037 ±0.0106	0	—	(−)	(−)	(−)	(−)	(−)
After dosing for 4 weeks	control	6.5 ±0.30	109 ±54.6	1.043 ±0.0027	0	trace	(−)	(−)	(−)	(−)	(−)
	50	6.7 ±0.23	95 ±82.2	1.047 ±0.0061	0	trace	(−)	(−)	(−)	(−)	(−)
	100	6.8 ±0.45	126 ±42.8	1.042 ±0.0026	0	trace	(−)	(−)	(−)	(−)	(−)
	200	7.0 ±0.20	114 ±10.0	1.033 ±0.0127	0	trace	(−)	(−)	(−)	(−)	(−)
	400	6.8 ±0.35	117 ±49.4	1.036 ±0.0124	0	trace	(−)	(−)	(−)	(−)	(−)

(−) : not detected

Table 6 Macroscopic findings

Tissues	Macroscopic findings	The number of dogs observed				
		Dose (mg/kg/day)				
		control	50	100	200	400
Aorta						
Trachea						
Heart						
Lung	Right lobe marbled with slight congestion	1				
Lymph node						
Liver	Surface slightly marbled with minimal congestion					1
Gall bladder						
Spleen	Ventral surface irregular (not smooth) dorsal surface less irregular			1		1
Pancreas						
Kidney	Left kidney slightly flaccid	1				
Urinary bladder	Minimal congestion at junction with urethra		1			1
Uterus						
Gonad						
Thyroid						
Adrenal						
Salivary gland						
Oesophagus Stomach Duodenum						
Jejunum and Ileum	Minimal generalised congestion throughout on mucosal surface	1		1		1
Caecum						
Colon	Minimal congestion at ileo-caecal valve				1	
Skin						
Skeletal muscle						
Mammary gland						
Tongue						
Brain						
Pituitary	Anterior cyst present		1			
Genitalia	Oestral changes present			1		
Sciatic nerve						
Injection site right foreleg	Moderate subcutaneous haemorrhage	3				2
	Slight subcutaneous haemorrhage		2	3	3	1
left foreleg	Slight subcutaneous haemorrhage	1	3	2	3	1
	Mederate subcutaneous haemorrhage	2				2

Table 7 Organ weights (g)

(mean±S. D.)

Dosage (mg/kg/day)		Control	50	100	200	400
Body wt.		11,500±1,900	11,700±1.530	11,000±2,470	10,900±1.190	10,300±290
Brain		77.1±3.18	76.1±5.10	76.6±1.39	73.1±1.03	77.3±0.85
Pituitary (mg)		70±12	70±6	60±6	70±3	80±12
Heart		89.0±7.83	95.6±2.46	86.2±10.60	91.6±6.71	88.0±4.65
Lungs		95.6±10.16	88.8±12.68	98.0±20.91	92.3±14.53	84.4±1.04
Liver		404.0±124.76	409.7±50.60	371.0±81.19	377.3±62.01	346.0±9.54
Spleen		60.5±6.07	59.0±18.45	46.3±14.97	44.4±13.87	53.5±23.37
Pancreas		24.2±8.30	20.6±4.45	24.2±4.10	22.6±5.15	28.9±4.55
Thymus		24.3±1.08	21.7±1.89	28.0±6.67	25.7±8.43	20.4±16.11
Uterus		4.02±1.229	3.83±1.883	12.01±11.818	3.30±0.509	3.41±1.268
Kidneys	L	30.6±5.09	32.0±1.47	27.4±6.53	30.1±3.32	29.3±2.12
	R	29.4±5.69	32.7±3.35	27.5±5.81	30.0±2.86	29.0±0.70
Thyroids	L	0.55±0.070	0.55±0.157	0.51±0.146	0.44±0.062	0.39*±0.035
	R	0.51±0.101	0.54±0.176	0.62±0.302	0.47±0.095	0.43±0.070
Adrenals	L	0.83±0.035	0.68±0.093	0.71±0.107	0.84±0.114	0.74±0.192
	R	0.83±0.082	0.68±0.115	0.75±0.139	0.73±0.050	0.74±0.045
Gonads	L	0.50±0.156	0.38±0.100	0.60±0.150	0.50±0.142	0.42±0.044
	R	0.45±0.146	0.42±0.147	0.78±0.524	0.41±0.105	0.37±0.040

* significantly different from control $P<0.05$

Table 8 Organ weights expressed as a percentage of the body weight (%)

(mean±S. D.)

Dosage (mg/kg/day)		Control	50	100	200	400
Brain		0.68±0.090	0.66±0.647	0.72±0.145	0.48±0.073	0.75±0.023
Pituitary		0.00064 ±0.000046	0.00057 ±0.000031	0.00060 ±0.000137	0.00064 ±0.000095	0.00075 ±0.000125
Heart		0.78±0.092	0.83±0.102	0.80±0.095	0.85±0.039	0.86±0.067
Lungs		0.85±0.187	0.76±0.038	0.90±0.043	0.85±0.039	0.82±0.030
Liver		3.48±0.553	3.56±0.688	3.39±0.05	3.46±0.267	3.37±0.005
Spleen		0.54±0.098	0.50±0.107	0.42±0.042	0.41±0.139	0.52±0.230
Pancreas		0.21±0.065	0.18±0.024	0.23±0.062	0.21±0.057	0.28±0.049
Thymus		0.21±0.027	0.19±0.015	0.26±0.080	0.23±0.066	0.20±0.150
Uterus		0.037±0.014	0.035±0.022	0.115±0.112	0.031±0.006	0.033±0.012
Kidneys	L	0.27±0.003	0.28±0.033	0.25±0.032	0.28±0.040	0.29±0.013
	R	0.26±0.010	0.28±0.031	0.25±0.045	0.28±0.036	0.28*±0.005
Thyroids	L	0.005±0.000	0.005±0.001	0.005±0.001	0.004±0.001	0.004*±0.000
	R	0.004±0.001	0.005±0.001	0.006±0.002	0.004±0.001	0.004±0.001
Adrenals	L	0.007±0.001	0.006±0.000	0.007±0.001	0.008±0.002	0.007±0.002
	R	0.007±0.001	0.006±0.000	0.007±0.002	0.007±0.001	0.007±0.001
Gonads	L	0.006±0.001	0.003±0.001	0.006±0.002	0.005±0.002	0.004±0.001
	R	0.004±0.001	0.004±0.001	0.007±0.005	0.004±0.001	0.004±0.000

* significantly different from control $P<0.05$

Table 9 Microscopic findings

Tissues	Microscopic findings	The number of dogs observed				
		Dose (mg/kg/day)				
		control	50	100	200	400
Aorta						
Trachea						
Heart						
Lung	A focal granulomatous reaction : a foreigen body is visible in the centre of the lesion		1			
	Minimal peribronchiolar lymphoid hyperplasia		1	1		1
	Focal interstitial pneumonitis		1	1		
Thymus						
Lymph node						
Liver	A minimal degree of generalised hepatocytic rarefaction	1	1			
	Minimal parenchymal chronic inflammatory cell infiltration with oil Red O no fat is seen					1
	Minimal focal periportal acute and chronic inflammatory cell infiltration. With Oil Red O is confined to bile duct epithelium					1
Gall bladder						
Kidney	Unilaterally, agranuloma (possibly parasitic in origin)	1				
	A focal area of interstitial nephritis with associated fibrosis in one kidney and minimal focal interstital nephritis in the other kidney	1				
	Small focal areas of dystrophic mineralisation in the renal papilla		1			
	Minimal interstitial nephritis with collections of chronic inflammatory cells beneath the epithelium of the renal pelvis		2			
Urinary bladder	Focal submucosal chronic inflammatory cell aggregates	1	1			
Uterus						
Gonad						
Thyroid Parathyroid	A developmental cyst is present in one parathyroid gland section					1
Adrenal						
Salivary gland						
Oesophagus Stomach Duodenum						
Jejunum						

Ileum	A small focal area of submucosal chronic inflammation					1
Small intestine	Small crypt abscess	1				
	A focal area of chronic inflammatory cells in the muscularis		1			
Colon Caecum						
Skeletal muscle						
Skin			1			
Mammary gland	Focal areas of peri-appendageal chronic inflammatory cell infiltration					
Injection site right foreleg	Minimal or moderate vessel proliferation, phlebitis, interstitial infiltration of inflammatory cells	2	1	2	3	1
	Minimal or moderate interstitial haemorrhage and/or haemosiderin pigmentation	3	3	2	2	3
Injection site left foreleg	Minimal or moderate vessel proliferation, phlebitis, interstitial infiltration of inflammatory cells	2		3	3	2
	Minimal or moderate interstitial haemorrhage and/or haemosiderin pigmentation	3	2	1	3	3

(iii) Urinalysis

Before dosing commenced one dog due to receive 100 mg/kg/day and one due to receive 400 mg/kg/day produced urine specimens with specific gravities below our normally accepted lower limit of 1.035. However, examination of a further sample from the former dog 5 days later revealed a normal value. All other results were within normal limits.

After 4 weeks dosing one dog receiving 200 mg/kg/day and one receiving 400 mg/kg/day produced urine samples with specific gravities below our normal lower limit. This was also observed before dosing commenced in the latter dog. When further urine samples were examined 2 days later the former dog produced urine with a specific gravity of 1.039, but the dog receiving 400 mg/kg/day again produced urine with a low specific gravity.

Red blood cells were detected in the urine provided by one dog receiving 200 mg/kg/day. Examination of the centrifuged deposit from a further urine sample collected 2 days later confirmed this finding. On neither occasion was haemoglobin detected in the urine. All other results were within normal limits.

Macroscopic findings

One dog receiving 400 mg/kg/day showed apparent slight thickening of the cephalic vein at both injection sites. The right injection site of one dog receiving 200 mg/kg/day was similarly affected. Slight to moderate subcutaneous haemorrhage was seen adjacent to the cephalic vein injection sites of most dogs from all groups, including the control group (Table 6).

Organ weights

The weights of the principal organs have been recorded both in grams and expressed as a percentage of bodyweight in Table 7 and 8.

The livers of one control dog and one receiving 50 mg/kg/day represented marginally in excess of 4% of bodyweight, which is our normally accepted upper limit. All other organ weights were within normal limits.

Microscopic findings

The results are presented in Table 9.

Lungs : A small number of dogs showed minimal evidence of chronic inflammatory disease with peribronchiolar or perivascular lymphoid hyperplasia.

Liver : No group-related changes were found. In one control dog and one receiving 50 mg/kg/day there was minimal degree of generalised hepatocytic rarefaction which was associated with a marginal increase in liver weight. In one dog receiving 400 mg/kg/day small areas of parenchymal chronic inflammatory cell infiltration

were noted. Such changes are occasionally seen in the livers of laboratory dogs. With special stains the distribution of glycogen was found to be normal in all animals and no abnormal fat deposits were detected.

Kidneys : A small number of dog showed minimal evidence of chronic interstitial nephritis with or without an associated fibrosis. In one kidney of a control dog a small parasitic granuloma was identified in the cortex.

Such changes are commonly observed in laboratory dogs.

Injection sites : A variety of histological changes were seen in the vein walls and surrounding connective tissues of the majority of the dogs examined. The overall nature and severity of the histological changes were similar in all groups. In almost all animals some degree of haemorrhage and haemosiderin pigment formation was found in connective tissues surrounding the vein wall. This haemorrhage was confined to the subcutaneous tissues and did not significantly extend into the overlying dermis or epidermis. In some animals there was associated acute and chronic inflammatory cell infiltration. In a small number of animals a minor degree of phlebitis, or rarely thrombophlebitis was present in the injected vein.

Occasionally, some degree of endothelial cell proliferation was noted, either in the intima or from small vessels on the adventitial aspect. These changes were considered to represent a pathological reaction to the trauma of the needle passage during venepuncture.

With regard to all other tissues, no abnormalities were found that could be related to treatment with the test material.

Discussion

A subacute toxicity study with Viccillin S was carried out using female Beagle dogs. Viccillin S was intravenously injected at dosage levels of 50, 100, 200 or 400 mg/kg/day for 30 days.

There were no deaths. Occasional vomiting in the group receiving 400 mg/kg/day, vasodilation and increased heart rate in the groups receiving 200 or 400 mg/kg/day and slight relaxation of the nictitating membrane in animals from all treatment groups were observed.

So, the following experiments were carried out to clarify the possible relationship between these clinical signs and the drug administration using Beagle dogs or cats by single intravenous injection:

(1) Vomiting

When 400 mg/kg of the drug in 20% solution was given to 4 dogs at the rate of 5 ml/minute, no dogs vomited. When it was administered at the rate of 10 ml/minute, 5 in 13 dogs vomited.

From this it might be concluded that infusion speed was related to vomiting.

(2) Relaxation of the nictitating membrane

By the intravenous administration of 400 mg/kg to 5 cats, the contractile response of the cat's nictitating membrane was suppressed by 10 to 75% (mean 39.6±29.0%) against electrical stimulation of the preganglionic fibre of the upper cervical sympathetic nerve. Similar reaction was obtained on the postganglionic fibre.

These results suggest that the slight relaxation seen in the dogs was not caused by ganglion block.

(3) Circulatory system[2)]

The effects of infusion speed and drug concentration on the circulatory system were studied using Beagle dogs. When the dogs received 200 mg/kg of Viccillin S at the rate of 2.75 ml/minute at a concentration above 5%, the heart rate and blood flow volume increased in proportion to the solution concentration, and the blood pressure fell inversely. In addition, the same changes were seen with an increase in infusion speed.

However, there were no effects on dogs receiving 200 mg/kg using a 1% solution at the rate of 2.75 ml/minute.

These studies indicated that the clinical signs such as vomiting and increased heart rate in the subacute toxicity study were mostly related to drug concentration or the infusion speed.

In the histopathological findings, inflammatory changes were found in and around the injection sites of most animals. The nature and severity of these signs were similar in all groups, so that they do not seem to be drug actions. Other histological canges were as follows : peribronchiolar or perivascular lymphoid hyperplasia, small area of interstitial pneumonitis or focal aggregates of chronic inflammatory cells in the lung, small areas of parenchymal chronic inflammatory cells in the liver and mmimal evidence of chronic interstitial nephritis. These slight changes are commonly seen in laboratory maintained dogs and both dose response relationship and biochemical analysis did

not reveal any relationship to them. Therefore, they were considered to have no experimental importance.

From the results of the histopathological findings and laboratory examinations of blood and urine, 400 mg/kg/day could be estimated as the maximum non-toxic dose, although one dog receiving this level exhibited a slightly elevated SGPT level. It is reasonable to conclude that 200 mg/kg/day is the maximum non-toxic dose taking into consideration vomiting and peripheral vasodilation which appeared to be related to speed of injection and drug concentration. However, slight relaxation of the nictitating membrane was seen at all dosage levels. This did not appear to be due to ganglion blockade and the significance of this finding at all dosage levels is probably of minimal importance.

Summary

Viccillin S, an antibiotic mixture consisting of equal amounts of sodium ampicillin and sodium cloxacillin, was administered intravenously to female Beagle dogs for 30 days at the dosage levels of 50, 100, 200 or 400 mg/kg/day.

(1) Dogs receiving 200 or 400 mg/kg/day showed slight clinical signs, namely, vomiting, vasodilation and increase in heart rate, but these signs were probably related to the drug concentration or infusion speed.

Slight relaxation of the nictitating membrane was seen at all dosage levels.

(2) Histopathological findings and biochemical examinations of blood and urine indicated that there were no definite abnormal effects caused by the drug administration in all groups.

(3) It is concluded that the maximum non-toxic dose is 200 mg/kg/day when Viccillin S is injected intravenously to Beagle dogs for 30 days.

Reference

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