

DELAYED VOMITING INDUCED IN DOGS BY INTRAMUSCULAR DIGOXIN

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Conditions necessary to the use of intramuscular digoxin as a challenge of delayed digitalis vomiting were studied in dogs. The $ED_{50} \pm SE$ was 0.0566 ± 0.00405 and the $ED_{84} \pm SE$ was 0.070 ± 0.004 mg./kg. Intramuscular injection of the ED_{84} was followed by an emetic syndrome which began in 2 to 3 hours, consisted of 5 to 10 vomiting spells and 50 to 100 retching spells, and stopped at 8 to 10 hours after injection. The ED_{84} produced some bradycardia, anorexia and diarrhoea. Repetition of the ED_{84} at intervals less, but not greater, than 4 days produced a more marked vomiting syndrome. The emetic syndrome was the same in male as in female dogs, the same during the night as during the day, and the same at all seasons of the year. The onset of vomiting was delayed and the number of vomiting and retching spells reduced when food was given at any time from 12 to 16 hours before, to 1 to 2 hours after, injection of the ED_{84} . Excessive (72-hour) starvation lessened the vomiting syndrome, probably due to excitement. The vomiting syndrome was less marked when recorded by observers who were particularly friendly with the dogs, outside the period of recording, apparently due simply to the nearness of such persons.

THE pharmacological testing of new agents for anti-emetic activity requires screening in animals, usually dogs, against a series of emetic challenges¹. The list of emetics includes² apomorphine hydrochloride, morphine sulphate, hydergine, copper sulphate, lanatoside C, and sometimes vertical swing³. The lanatoside C is usually given intravenously to produce vomiting in about 10 minutes² or intraperitoneally to produce vomiting in about 50 minutes.

Gold and others⁴ have reported that when lanatoside C is given orally to cats, vomiting begins at intervals of from 4 to 8 hours (range 19 to 695 minutes). Borison⁵ found that ablation of the emetic chemoreceptor trigger zone prevented the early type of vomiting from intravenous cardiac glycosides in over 90 per cent of cats and the late or delayed type of vomiting from parenteral or oral glycosides in less than 50 per cent of cats. The difference may be calculated to be significant at $P < 0.001$. Borison and Wang⁶ conclude that cardiac glycosides may produce vomiting, "by acting at more than one receptor site".

Since the purpose of an anti-emetic screening programme is to challenge suitable animals against as many as possible of the known types of vomiting, it seemed desirable to include delayed vomiting induced by cardiac glycosides in such a programme. Digoxin was selected in our laboratory as the challenging agent for delayed digitalis vomiting. It was found in preliminary trials that a suitable dose of digoxin given

INDUCED VOMITING BY INTRAMUSCULAR DIGOXIN

intramuscularly would produce in dogs a vomiting syndrome beginning in 2 to 3 hours and ending in 9 to 10 hours after injection. A systematic study was then made of factors which might affect this vomiting syndrome. This work is reported below.

METHOD

The experiments were performed upon mongrel dogs of both sexes and 6 to 12 kg. body weight. The animals were housed in Wahmann LC93 dog cages, one dog per cage. They were fed Purina fox chow checkers, meat, bread, milk and water *ad libitum* and given supplementary Decavitamin Capsules, U.S.P. XV. They were observed for a period of at least one month to assure good health before being challenged with digoxin. Some dogs have a more pronounced vomiting syndrome than others to emetic challenges. In arranging a group for any study, an attempt was made to obtain a cross section or an "average" group in so far as susceptibility to vomiting was concerned.

In each dog there were recorded time in minutes to onset of vomiting, the number of vomiting and the number of retching spells, the duration of the vomiting syndrome, and the general clinical signs. The technique was that of Boyd and Boyd⁷.

Digoxin was used in the form of Digoxin Injection, B.P. 1958, and was obtained from Burroughs Wellcome and Company (Canada) Limited of Montreal.

RESULTS

FOURTEEN dogs were given at weekly intervals increasing doses of digoxin intramuscularly. They were arbitrarily given no food from 16 hours before injection to 8 hours after injection. Pertinent data are summarised in Table I. The median emetic dose \pm standard error (ED50 \pm SE) was 0.0566 \pm 0.00405 and the ED84 \pm SE was 0.070 \pm 0.004 mg./kg. From this experiment a dose of 0.07 mg./kg. intramuscularly was selected as the challenging emetic dose of digoxin. This dose is approximately one-tenth the intramuscular median lethal dose in the guinea pig per kg. weight⁸.

TABLE I
DOSE-RESPONSE DATA UPON 14 DOGS

Dose mg./kg. i.m.	Incidence of vomiting per cent	Interval to onset minutes mean \pm SE	Number of vomiting spells mean \pm SE	Number of retching spells mean \pm SE
0.03	0	—	0 \pm 0	0 \pm 0
0.04	0	—	0 \pm 0	0 \pm 0
0.05	43	193 \pm 8.3	0.93 \pm 0.32	7.0 \pm 3.1
0.06	71	197 \pm 16.7	2.64 \pm 0.59	28.7 \pm 7.1
0.07	86	158 \pm 13.4	4.85 \pm 0.86	43.4 \pm 12.4
0.08	93	156 \pm 14.7	5.29 \pm 0.99	57.1 \pm 13.1

The challenging dose of 0.07 mg./kg. produced a bradycardia which reached a maximal average of 22 per cent fewer beats per minute at 2 hours after injection. Cardiac rate returned to normal at 24 hours (Table II). During the first day the dogs ate less than usual, in spite

of the long fast, and lost some weight (Table II). A diarrhoea appeared during the second day. From the 3rd day on, the animals appeared clinically normal.

TABLE II
THE EFFECT OF THE CHALLENGING DOSE OF DIGOXIN ON HEART RATE,
BODY WEIGHT, AND FOOD INTAKE

Interval days	Heart rate per minute mean \pm SE	Weight kg. mean \pm SE	Food intake g. chow/kg./24 hr. mean \pm SE
0 (before)	87.4 \pm 6.8	9.13 \pm 0.56	26.7 \pm 2.6
1 (after)	89.5 \pm 7.2	8.99 \pm 0.55	12.6 \pm 2.2
3 (after)	81.5 \pm 5.8	9.31 \pm 0.62	29.2 \pm 2.3
5 (after)	85.3 \pm 6.6	9.16 \pm 0.60	22.8 \pm 3.4
7 (after)	88.3 \pm 6.1	9.22 \pm 0.56	22.1 \pm 2.8

The minimal necessary interval of rest between injections of challenging doses was determined in 12 dogs. They were given no food for 16 hours and injected intramuscularly with digoxin in a dose of 0.07 mg./kg. at intervals of 6, 4 and 2 days on days 0, 6, 10 and 12 respectively. The vomiting syndromes on days 0, 6 and 10 were insignificantly different from each other. The vomiting syndrome on the 12th day had a significantly ($P < 0.001$) greater number of vomiting and retching spells. Presumably enough of the challenging emetic dose of digoxin was present at 2 days to augment the effect of a further challenging dose given at that time. The results indicated that an interval of 4 to 7 days should be allowed between emetic challenges.

Influence of sex of the animal was determined upon 14 male and 18 female dogs. The syndrome produced by the challenging emetic was the same in male as in female dogs.

Influence of the time of day that digoxin was injected was determined upon 16 dogs. They were given digoxin at 8:00 a.m. and the syndrome measured during the hours of daylight on one occasion. On the second trial, they were given digoxin at 4:00 p.m. and the syndrome followed until past midnight. The vomiting syndrome in one group did not differ significantly from that in the second group.

Influence of season was measured in 48 dogs, given 0.07 mg./kg. of digoxin intramuscularly in October, 1957, and in January, May and July, 1958. The vomiting syndrome was the same on all four occasions.

What might be called the personality of the observer was found to be a factor influencing the intensity of the vomiting syndrome in the dogs. Four different observers recorded the vomiting syndrome on repeated occasions in 14 dogs given a challenging dose of digoxin. Each observer was well known to the dogs from frequent previous recordings. The observation itself was carried out in the same manner by each observer who sat at some distance from the cages and did not speak to or in any way handle the animals after injecting digoxin. The vomiting syndrome was ($P < 0.001$) less as observed by two persons than by the two other persons. The only apparent difference was that the two former observers were what might be called friendly with dogs while the two latter observers paid little attention to the dogs apart from the actual

INDUCED VOMITING BY INTRAMUSCULAR DIGOXIN

experiment. Because of this, each factor was worked out entirely by at least one observer and results of one observer were not otherwise compared with results of a second.

The time of feeding influenced the severity of the vomiting syndrome. When this was discovered, it was systematically investigated by removing food from dogs at 48 hours before injection of a challenging dose of digoxin. This made the animals hungry and when offered a standard meal at the intervals noted in Table III, they ate it quickly. As shown by data summarised in Table III, the onset of vomiting was delayed and the number of vomiting and retching spells reduced when food was given any time from 12 to 16 hours before to 1 to 2 hours after injection of digoxin. These results suggest that some substance produced during digestion of food, possibly by the liver, decreases the sensitivity of the medullary areas concerned to the emetic action of digoxin.

TABLE III
THE VOMITING SYNDROME AS AFFECTED BY TIME OF FEEDING

Time of feeding-hr. after or before digoxin	Incidence of vomiting per cent	Minutes to onset mean \pm SE	Number of vomiting spells mean \pm SE	Number of retching spells mean \pm SE
10 (after)	98	146 \pm 6	7.6 \pm 0.6	68 \pm 8
2 (after)	92	175 \pm 13*	7.3 \pm 1.7	57 \pm 16
1 (after)	50**	212 \pm 15**	4.2 \pm 0.5**	20 \pm 9**
0	60**	216 \pm 9**	3.3 \pm 0.6**	13 \pm 3**
1 (before)	67*	223 \pm 8**	3.1 \pm 0.9**	21 \pm 8**
2 (before)	78	198 \pm 18**	4.1 \pm 1.6*	9 \pm 4**
4 (before)	62**	212 \pm 9**	2.6 \pm 0.6**	15 \pm 8**
6 (before)	56*	168 \pm 20	1.0 \pm 0.4**	8 \pm 4**
8 (before)	80	201 \pm 12**	2.4 \pm 1.3**	13 \pm 8**
12 (before)	86	190 \pm 25	3.0 \pm 0.9**	19 \pm 10**
16 (before)	86	158 \pm 13	4.9 \pm 0.9*	43 \pm 12
24 (before)	100	156 \pm 23	7.3 \pm 1.2	99 \pm 18
48 (before)	100	150 \pm 12	9.4 \pm 1.1	75 \pm 18
72 (before)	90	171 \pm 5	2.6 \pm 0.5**	30 \pm 6**

* Probability (P) that mean difference from controls (10 after) equals zero \leq 0.05.

** Probability (P) that mean difference from controls (10 after) equal zero \leq 0.01.

The results indicated that a maximal emetic response to digoxin is obtained by giving the dogs no food between 16 to 48 hours before injection of the drug and the time that vomiting ceases. These conditions with a dose of 0.07 mg./kg. intramuscularly of digoxin caused vomiting to start during the 3rd hour, to reach and maintain a peak during the 3rd to 7th hours, and to gradually lessen to zero during the 8th to 10th hours.

When dogs were starved for 72 hours before giving digoxin, the vomiting syndrome was less marked than when starved for 48 hours (see Table III). After the injection of digoxin, the 72-hour-starved dogs, normally placid and readily handled, became excited, restless, irritable and occasionally vicious.

DISCUSSION

From the results described above, certain conditions have been established for the use of digoxin intramuscularly as a delayed digitalis emetic challenge in dogs. The dogs should be starved for 16 to 48 hours before injection. Food must be withheld further until at least 2 hours (and

is given most conveniently 10 hours) after injection of digoxin. The intramuscular ED84 of digoxin is 0.07 mg./kg. The vomiting syndrome begins during the 3rd hour and lasts to the 9th or 10th hour after injection. The syndrome consists of 5 to 10 vomiting spells and 50 to 100 retching spells. It is accompanied by bradycardia, some anorexia at the end of the syndrome, and some diarrhoea the next day. The dogs appear normal on the 3rd day but should not be used again for digoxin-challenge until at least the 4th day.

The sex of the dog, the season of the year, and the time of day digoxin is injected have no influence upon the vomiting syndrome. The vomiting syndrome is reduced if the attention of the dogs is diverted by the nearness of a recorder who is friendly with them. The vomiting syndrome is also reduced when the dogs become restless and irritable from prolonged (3 day) starvation.

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REFERENCES

1. Boyd, *Pharmacology in Medicine*, 2nd Edn., McGraw-Hill, New York, 1958, chapter 46, p. 694.
2. Wang, *J. Pharmacol.*, 1958, **123**, 306.
3. Boyd, *Canad. med. Ass. J.*, 1957, **76**, 286.
4. Gold, Cattell, Modell, Greiner and Guevara, *J. Pharmacol.*, 1950, **98**, 337.
5. Borison, *ibid.*, 1952, **104**, 398.
6. Borison and Wang, *Pharmacol. Rev.*, 1953, **5**, 193.
7. Boyd and Boyd, *Canad. J. med. Sci.*, 1953, **31**, 320.
8. Spector, *Handbook of Toxicology*, Volume I, Saunders, Philadelphia, 1956, p. 104.