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CLIOQUINOL TOXICITY IN THE DOG

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SUMMARY

A number of instances have been reported in the scientific literature in which acute intoxication with halogenated oxyquinolines has led in some species to convulsions, often followed by death. The toxicity of repeated doses of clioquinol has been investigated extensively in the dog. The clinical syndrome induced in this species is characterized by anorexia, weight loss, extreme muscle weakness and emaciation. In some animals surviving this impairment of condition for several weeks, neuropathy of the central nervous system, but not of the peripheral nerves ensued. It is suggested that these toxicological manifestations are less dependent on the dose-level than on the degree of absorption. Some suggestions regarding the aetiology of the lesions are made.

INTRODUCTION

Acute intoxication after treatment with clioquinol in dogs with diarrhoea was reported in 1965 by Hangartner [1]. Similar findings were described by Schanz and Wikström in the same year [2], and a full account of these and further observations was published in 1973 and 1974 by Lannek and her co-workers [3–7]. The case histories showed that the side effects provoked by various halogenated oxyquinolines in dogs consisted of an acute syndrome, characterized by hyperexcitability and convulsions and also by disturbances in other organs besides the central nervous system (CNS) indicative of severe systemic toxicity. The clinical findings were confirmed

Abbreviations: CNS, central nervous system; CPK, creatine phosphokinase; SAP, serum alkaline phosphatase; SGPT, serum glutamic pyruvictransaminase; SMON, subacute myelo-optic neuropathy.
in a number of dogs in which oxyquinoline intoxication was induced experimentally [5]. The occurrence of severe symptoms appeared to be conditional on an extremely high degree of absorption from the gut. It was demonstrated that the intestinal absorption of labelled clioquinol was greatly increased when the dogs had not been fasted. Under these conditions, high peak concentrations occurred in the plasma, but the distribution of the substance in the tissues was not changed [6]. The consumption of fat was considered to be the significant factor. An influence of the nutritional state on the degree of absorption has also been reported by Chen et al. [29]. In 3 cases of severe experimental intoxication, morphological examination revealed the presence of acute cellular lesions, mainly in the hippocampus but also in other parts of the CNS (thalamus, hypothalamus and amygdala); no changes were detected in the optic tract or in the peripheral nerves [7].

The lesions that were produced were ascribed by the authors to what may be called an acute encephalopathy consistent with the severe clinical symptoms observed, predominantly comprising cardiac insufficiency and liver damage.

In toxicity tests in Beagle dogs, Hess et al. [8] had shown that the dogs tolerated daily oral treatment with 100 mg clioquinol/kg of body weight for a period of 4 months without suffering any adverse effects. Of the group of 6 animals treated daily in this study with 300 mg/kg, two died within the first 2 weeks and a third had to be killed after 3 months on account of its poor general condition. No specific organ damage was demonstrated. Thomann et al. [9] compared the toxic effects of acrylamide with those of clioquinol and found that dogs treated with 5 mg/kg of acrylamide for 9 weeks clearly exhibited specific neurotoxic effects, whereas dogs treated continuously for 12 months with 200 mg/kg of clioquinol showed no evidence of neuropathy: despite this finding, the possibility that neuropathy might occur in this species under certain conditions following clioquinol treatment was not entirely dismissed [12].

In a series of experiments carried out in Japan, Tateishi et al. [13-16] attempted to demonstrate the nature of optico-myeloneuropathy in animals treated with clioquinol. These studies were conducted under widely varying experimental conditions. The dogs were usually started on moderate doses, and these were increased gradually until severe signs of general toxicity appeared. At this stage, the dose of clioquinol was either maintained at the same level for as long as possible or reduced enough to assure survival of the animals. The dogs consequently remained in a very poor state of health and, besides suffering liver and kidney damage, often developed neurological symptoms, such as paresis and partial loss of pain perception in the hind legs.

It was stated that there were lesions in the peripheral nerves and degenerative changes, predominantly of the long sensory fibres, and also in the dorsal spinal ganglia and in the ganglia of the autonomic nervous system. The pattern was said to conform to the diagnostic criteria laid down for the human disease "subacute myelo-optic neuropathy" (SMON). This neurological disorder encountered in Japan, for which a causal relationship to
Clioquinol has been alleged [10,11], has been characterized as a distally distributed type of neuropathy mainly involving sensory pathways but affecting motor neurons as well [17]. Evidence of peripheral neuropathy appeared equivocal [18] and, in fact, Tateishi et al. were unable to confirm their initial findings. In their latest publication [19], they conclude that in Beagle dogs no significant lesion was detected in peripheral nerves or dorsal root ganglia.

Neurohistological studies of the CNS disclosed lesions located particularly in the cervical part of the dorsal ascending pathways of the spinal cord, but occasionally also in the optic tract and optic nerve and in certain regions of the brain. The severity of these lesions appeared to be related to the duration of the state of emaciation produced by general intoxication. Animals that did not develop severe and long-lasting symptoms of intoxication had no significant neuropathological lesions [13–16,18,19].

In a particular experiment in which Beagles were given clioquinol in daily oral doses rising gradually by increments of 50 mg/kg to a maximum of 600 mg/kg daily, Hess et al. [20] found no signs of neuropathy. In some dogs receiving very high doses, however, non-specific signs of toxicity were produced. In a further series of experiments [21], Entero-Vioform (powder consisting of 93% clioquinol and 7% sapamine) was given in doses raised gradually by increments of 50 mg/kg body weight/day to a maximal daily dose of 1000 mg/kg with the aim of exceeding the tolerated dose and producing lethal intoxication. It was found that in animals kept alive after convulsive episodes, or after a serious loss of general condition had occurred, secondary changes in the CNS became apparent: in 7 out of 26 treated dogs detailed neuropathological examination of the central and peripheral nervous system revealed lesions of an acute dystrophic type in the optic nerve and the optic tract, and in 5 of these animals also in the fasciculus gracilis of the spinal cord. However, extensive histological study of the peripheral nerves, of the spinal ganglia and of parts of the autonomic nervous system revealed no pathological changes. The results of this study indicated that the lesions observed at toxic dose-levels were related neither to the daily dose nor the total amount of Entero-Vioform ingested, nor to the duration of treatment. Thus changes in the central nervous tissue occurring in the dogs that had convulsed and/or were at the point of death were considered secondary to the severe disturbance of the whole organism (possibly of circulatory nature or due to lack of essential nutrients) and not directly due to the action of the product administered. The findings were interpreted as indicating that no specific neurotoxicity had occurred.

Heywood et al. [22] administered clioquinol at dose-levels of 100, 250 and 400 mg/kg daily for 6 months. At 100 mg/kg no adverse effects were noted. At 250 and 400 mg/kg daily body weight gain was suppressed and two dogs receiving the high dose had to be killed in a debilitated condition for humane reasons. Three dogs on 400 and one dog on 250 mg/kg showed signs of hepatotoxicity, and upon microscopical examination a small number of vacuolated and occasionally degenerative hepatocytes were found. The
authors also reported neurological disturbance and pathological changes in the posterior columns of the spinal cord in the dogs receiving dose-levels of 250 and 400 mg/kg daily.

The present paper describes further experiments in Beagles in which clioquinol was administered daily over a period of 25 weeks under similar experimental conditions.

MATERIAL AND METHODS

Nine male and 9 female Beagles were allotted to 3 groups: group 1 received empty gelatine capsules and acted as control; group 2 was given 250 mg; and group 3 400 mg of pure clioquinol/kg of body weight/day. The test compound was administered undiluted in gelatine capsules, daily for 25 weeks. The dogs were housed singly and were initially offered 400 g of a dry diet (Spratt’s Dog Diet P62, Spillers Ltd., Barking, Essex) each morning; 200 ml cows’ milk was offered, on week-days only, throughout the experiment. Water was freely available.

To prevent anorexia and marked loss of condition and to keep the animals alive as long as possible, the diets were varied by moistening with water, by the addition of canned dog-meat (Lassie, Pedigree Petfoods, Melton Mowbray, Leics.) or by offering canned dog-meat alone.

Throughout the trial, clinical signs were recorded daily. Body weight was recorded 3 times a week. Ophthalmoscopic and neurological examinations were carried out before the start of the experimental period and at regular intervals thereafter. At the beginning, and after 4, 8, 12, 17 and 24 weeks of drug administration, blood samples were taken and the following parameters measured by standard techniques: haemoglobin, haematocrit; erythrocyte, total leucocyte, reticulocyte, thrombocyte and differential white cell counts; prothrombin time; blood glucose and urea; serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SAP), total protein and electrophoresis of serum proteins and electrolytes (Na⁺, K⁺). At the same intervals, urine samples were obtained for complete urine analysis, including examination of sediment. In addition to these routine tests, creatine and creatinine excretion, serum creatine phosphokinase (CPK), magnesium, calcium, chloride, bicarbonate and phosphorus, red cell and plasma cholinesterase and riboflavin excretion were estimated at intervals during the dosage period.

At the end of the test period — or, in 4 cases, after prior termination for humane reasons — the animals were killed and the organs examined macroscopically. The major organs were weighed, and pieces of these and other tissues were removed and prepared for histological examination. Particular attention was paid to the muscular and nervous systems: sections of the vastus medialis, gluteus medius and biceps femoris muscles were taken from the right hind limb, and longitudinal and transverse sections were prepared. Besides the routine sections of the brain and spinal cord, special preparations were made of autonomic ganglia and the proximal sciatic, lateral cutaneous
femoral, tibial and fibular (at the level of the talus and calcaneum respectively) nerves. Additional sections were cut to show the brain stem, in particular the distal medulla oblongata and the contiguous cervical spinal cord, and representative spinal ganglia of the cervical, thoracic and lumbar regions. The sections were stained with haematoxylin and eosin and also with special stains (Luxol fast blue, cresyl violet, Glees-Marsland, Bodian, Bender-Spielmeyer) allowing selective visualization of the myelin sheaths and axonal cylinders.

RESULTS

Four dogs were killed for humane reasons before the full course of treatment had been administered. One male from group 3 (400 mg/kg daily) was killed on day 35 and two others from this group were killed on day 116. A male from group 2 (250 mg/kg daily) was killed on day 88. These animals had all displayed general deterioration of their condition, abnormal gait with partial or complete hind limb paralysis, yellowing of the hair and patchy hair loss. On histological examination of the fasciculus gracilis of the spinal cord of the animals killed on days 35 and 88 some vacuolation of this area was observed; however, no unequivocal neuropathy could be detected. The two dogs killed on day 116 showed degenerative changes in the fasciculus gracilis of the cervical spinal cord that are dealt with in more detail below. Loss of condition, abnormal gait, yellowing of the hair and hair loss were the clinical signs noted in both groups of animals dosed for 25 weeks. Physical development was retarded compared with that of normal whelps of the same age.

From week 3, 3 animals receiving 250 mg/kg daily and 5 animals receiving 400 mg/kg daily showed marked loss of condition. This was particularly noticeable in the animal receiving 400 mg/kg that was killed on day 35.

All the dogs were gaining weight satisfactorily before the start of the study, and no severe adverse effects were seen when the test compound was first administered. Subsequently, body weight gain was suppressed in the dogs receiving 400 mg/kg daily. Individual animals on 250 mg/kg daily showed episodes of depressed body weight gain and/or weight loss, alternating with periods of normal development.

Dogs in both treatment groups showed loss of appetite from the first week of dosing. It is usual practice in toxicological studies to feed a standard diet; however, as one of the objectives of this study was to observe dogs surviving the generally toxic effects of treatment for as long a period as possible, tinned meat was added to the diet in order to prolong the life of the animals. Despite this modification to the diet, the intake of essential nutrients and trace elements was in fact reduced in some dogs over a prolonged period of time, and the resultant malnutrition was correlated with the reduced body weight gain.

Abnormal gait was seen in all dosed animals by day 57. The hind limbs only were involved. Those animals least affected showed slight incoordination of the hind limbs with some splaying to maintain stance. The most
The severely affected dog completely lost the ability to use its hind limbs though some muscle tonus was still present. The degree of change varied, in that most dogs surviving the experimental period were at their worst during weeks 16–18, but by the end of the experimental period many showed only minor disturbances in gait. No group-related pattern was evident. Neurological examination revealed disturbance of the placing and postural reactions in all the animals in group 3 and some in group 2. The reflex reactions and responses considered abnormal were those concerned with hind limb proprioception, but no abnormalities were detected in the forelimbs. The neurological disturbances were not consistent, but varied from examination to examination. Yellow staining of the hair was first observed on day 17 in the females of group 2. This sign was subsequently noticed in animals from both dosed groups, and by day 29 most dosed dogs were showing some degree of yellowing of the hair, particularly on the chest and forelimbs. Chemical analysis by gas chromatography showed this yellowing to be due to staining with unchanged clioquinol, presumably deriving from the animals’ urine.

Ophthalmoscopic examination revealed paleness of the optic papilla in one female dog receiving the high dosage. The pupillary light reflex was found to be sluggish.

Haematological monitoring showed that the red cell counts tended to be lower in the dosed groups than in the controls. This was noted after 4 weeks of administration of the test compound and persisted till the end of the study. The only other abnormality detected as a result of laboratory investigations that could have been attributable to treatment was a higher mean urinary creatine: creatinine ratio in the dosed groups than in control animals, indicative of a disturbed muscle function. This was found after 12, 18, 20 and 22 weeks’ administration of the test compound, but after 24 weeks the means were similar in all groups.

Macroscopic post-mortem examination revealed atrophy of the limb musculature in 3 females in group 3 that had survived the entire 25-week treatment period; this muscle wasting was associated with general debility and attributed to treatment with the test compound.

The absolute organ weights were considered to be within normal limits. However, in the dogs of groups 2 and 3, the mean liver weight expressed as a percentage of the body weight was significantly greater than the control values ($P < 0.05$), a not infrequent finding in toxicity studies [35].

Histological changes were identified in the dorsal columns of the spinal cord in one dog receiving 250 mg/kg daily and in 4 dogs receiving 400 mg/kg daily. In one of the dogs given the high dose the changes were present at various levels of the cord, whereas in the dog receiving 250 mg/kg daily they were restricted to the cervical and thoracic regions, and in the 3 remaining high dose-level animals (two of which were the dogs killed on day 116) the cervical region only was affected. In these last-mentioned animals, the changes were minimal and located close to the nucleus gracilis of the medulla. The lesions were of a dystrophic nature, probably acute in onset and were
Figs. 1–3. Cervical Goll’s tract.

Fig. 1. Silver stain. Disseminated fragmentation of the axons.

Fig. 2. Luxol blue stain. Break-down products of the myelin sheaths are visible in form of Luxol-positive droplets.

Fig. 3. H-E stain. Degenerating nerve fibres exhibit “digestion chambers” (vacuoles) occasionally enclosed by scavenger cells.

Fig. 4. Peripheral (sciatic) Nerve. H-E stain. The normal histological structure of the peripheral nerve is evident.

All the figures: female dog No. 752 (400 mg/kg/day), longitudinal paraffin sections. (Original magnification ×400).
Fig. 5. Optic nerve. (Original magnification × 800). Axonal swelling and degeneration.

Fig. 6. Optic nerve. (Original magnification × 100). Axonal swelling and degeneration.
Fig. 7. Anulospiral endings in flexor muscle of 5th digit. Sudan black B stain. Normal histological structure preserved. (Original magnification X 200).

not considered to be due to any ageing process. The morphological features included axonal swelling and degeneration, myelin breakdown with myelophagia and occasional astrocyte activation (Figs. 1—4). Similar changes, including axonal swelling, were seen in the optic nerve of one dog (Figs. 5, 6). Although the peripheral nerves and the spinal and autonomic ganglia were examined thoroughly, no pathological changes were detected. Nor were any pathological changes detected in sections taken from the flexor muscle of the 5th digit and stained with Sudan black B [17]. The myelin sheaths were preserved and the muscular motor and anulospiral endings were demonstrated (Fig. 7).

DISCUSSION

A number of instances have been reported in the scientific literature in which acute intoxication with halogenated oxyquinolines has led in some species (mouse, cat, dog) to convulsions, often followed by death.

Clinical signs of acute, non-specific encephalopathy after the administration of lethal doses of a wide variety of drugs and other chemical agents are well known. Severe central nervous symptoms, including ataxia, prostration and convulsions, are produced and may conform to a more or less distinct pattern of structural change [23]. Some types of lesion, usually associated with convulsive states, have been shown to affect the hippocampus. They are
considered to be of an ischaemic nature, involving parts of the brain tissue particularly susceptible to decreased supply of oxygen [34]. Changes in the hippocampus of dogs were observed by Lannek and Jönsson [7] after the administration of acutely toxic doses of clioquinol. The various aspects of toxic encephalopathy in mice and in dogs produced by different kinds of convulsive treatment and involving damage to the hippocampus have been described [24]. A further type of effect of experimental poisoning with clioquinol has been observed exclusively in dogs. The clinical syndrome induced in this species is characterized by poor appetite, weight loss, extreme muscle weakness and emaciation. Convulsive seizures may occur at any time during exposure to high doses of clioquinol; such episodes are sometimes followed by death. If this impairment of condition is survived for several weeks, neuropathy of the CNS may ensue.

During the present study, changes were made in the animals' diet in an attempt to maintain their condition and ensure that they received the test compound in an unfasted state. It was also the practice to offer 200 ml of fresh cows' milk, on week-days only. It was not unusual to observe by Monday morning some remission of clinical signs that had been pronounced before the week-end. This may have been attributable to facilitation of the absorption of test compound on week-days by the presence of milk fat, since milk was readily taken even by animals in a debilitated condition, a factor which would be in accord with the observations of Lannek and Lindberg [5,6], and with the findings of Heywood et al. [22]. Additional evidence that dietary factors might exert an influence on the absorption of clioquinol comes from the earlier studies carried out by Japanese and Swiss workers. Tateishi et al. [13,14,18] administered clioquinol suspended in milk, whilst Hess et al. [8,9,20,21] gave the compound in gelatine capsules to dogs in a fasted state.

In the present experiment the limits of tolerance were exceeded in both dose-groups; in the high dose group all 3 males and in the low dose group one male had to be killed in a dying state after periods ranging from 1 to 4 months. The animals showed a loss of condition, and later abnormal gait was seen, deteriorating in two animals into paralysis of the hind-quarters. The changes observed in the central nervous tissue occurred in the dogs that had shown episodes of sudden loss of condition, anorexia and weight loss. They were not clearly related to the dose or the duration of administration, but there was a significant correlation between the severity and duration of the deterioration of general health and the lesions observed.

It would therefore appear that the toxicity produced in dogs through the administration of clioquinol depended less on the actual dose than on the high degree of absorption. The lethal syndrome resulting in some animals was characterized by damage to various organ systems, in particular by hepatotoxicity and the development of anaemia, and also included an acute dystrophic change in isolated axons in the CNS.

There is also a close similarity between the foregoing lesions of the dorsal columns of the spinal cord, encountered in debilitated dogs and the neuro-
pathy observed in this species as a result of nutritional deficiency [25], as part of the gastrectomy syndrome [26], or following overdosage with vitamin B₆ [27]. In addition, granular lesions believed to represent degenerating axis cylinders have been observed in apparently normal dogs as a non-specific defect due to ageing [28].

The appearance of structural lesions observed in the CNS of dogs had not conformed with the diagnostic criteria of the Japanese disease entity “SMON” [15,30,31] as defined by Kono [10], or with the interpretation of the effects seen after clioquinol treatment as a specific basic peripheral neuropathy of the “dying back” type [17]. The lesions show a close similarity to those induced in rhesus monkeys by long-standing dietary vitamin B₁₂ deficiency [33]. In the course of a study in which Hess et al. [32] administered daily oral doses of 400 mg/kg of clioquinol to two groups of beagles that had either been fasted or been fed immediately beforehand, serum vitamin B₁₂ and serum folic acid concentrations were determined. In contrast to those found in fasted animals, B₁₂ levels in the pre-fed dogs were decreased and serum folate increased as a result of treatment. The metabolic situation created in the dog by the administration of clioquinol might provide a simple experimental model of nutritional disease of some relevance to man, including the consideration of certain features of SMON, of which SCD is the main neurological differential diagnosis [11].

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