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Thiacetarsamide (adulticide) *versus* melarsomine (RM 340) developed as macrofilaricide (adulticide and larvicide) to cure canine heartworm infection in dogs

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Summary — To implement a new macrofilaricide, treatment of heartworm infection or disease in dogs was checked in all the clinical situations *ie* from subclinical to severe disease. After preliminary toxicity and efficacy models on experimentally infected dogs, in addition, to the reference posology (2.5 mg of melarsomine (RM 340)/kg twice, 24 h apart by deep IM injection) a more practical program for vet practitioners was suggested (2.2 mg/kg twice, 3 h apart) using modelization of the pharmacokinetic data. The two treatments were equivalent as shown on models with experimental infection of dogs, critical tests on naturally infected dogs and clinical trials in veterinary practice. We advise using specific and well adapted therapeutic programs for each of the clinical classes (class 1: subclinical, class 2: moderate, class 3: severe). The safety margin is respectively x 3 or x 2.5 in contrast with thiacetarsamide which, being hepatotoxic, has no safety margin, and sometimes is nephrotoxic at the recommended dose. RM 340 is fully effective on *D immitis* adults (even on young ones of 7 months old) and L5 immatures (4 months old) when thiacetarsamide is poorly effective on 7 months or ineffective on 4-month-old parasites. Clinical trials in veterinary practice showed that the programs are well adapted to many clinical situations. The product is effective, relatively safe and easy to handle by IM injection. Preliminary results show its possible use as tactical treatment (2.2 mg/kg twice, 3 h apart) twice a year in mid August and December-January to prevent heartworm disease.

melarsomine / RM 340 / thiacetarsamine / *D immitis* / prevention / treatment

Résumé — Comparaison de thiacétarsamide, (adulticide) et de mélarsomine (RM 340), un macrofilaricide (adulticide et larvicide) pour le traitement des infestations du chien par *Dirofilaria immitis*. Pour développer un nouveau macrofilaricide, le traitement des infestations par *D immitis* et des pathologies dues au parasite (dirofilariose) a été mis au point dans toutes les situations cliniques rencontrées : dirofilariose sub-clinique, modérée ou sévère. Après des essais préliminaires de toxicité et d'efficacité sur modèle d'infestation expérimentale à une posologie de référence (2,5 mg de mélarsomine (RM 340)/kg, 2 fois à 24 h d'intervalle par voie intramusculaire profonde), un programme plus pratique en clientèle vétérinaire a été proposé (2,2 mg/kg 2 fois à 3 h d'intervalle) après modélisation des résultats de pharmacocinétique. Les 2 posologies se sont montrées équivalentes sur modèle d'infestation expérimentale et sur chiens à infestations naturelles (essais critiques et essais cliniques), en clientèle vétérinaire. Nous recommandons l'utilisation de programmes thérapeutiques adaptés à chacune des classes cliniques de dirofilariose rencontrées (classe 1 = sub-clinique; Classe 2 = modérée; Classe 3 = sévère). La marge thérapeutique est respectivement de 3 ou de 2,5 fois, ce qui se compare favorablement à la thiacétarsamide qui n'a pas de marge, étant

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hépatotoxique et parfois néphrotoxique à la posologie recommandée. Le RM 340 est complètement efficace sur D immitis adultes (même les formes jeunes de l'adulte, de 7 mois d'âge) et immatures : les L5 de 4 mois d'âge, stades parasitaires où la thiacétarsamide est partiellement efficace (7 mois) ou inefficace (4 mois). Des essais cliniques en clientèle vétérinaire ont montré que les programmes thérapeutiques étaient bien adaptés à la plupart des cas rencontrés. Le produit a été efficace, relativement sûr et facile à manipuler car les injections se font par voie intramusculaire. Des résultats préliminaires ont montré l'utilisation du RM 340 en traitement tactique 2,2 mg/kg 2 fois à 3 h d'intervalle 2 fois par an en août et décembre-janvier pour prévenir la pathologie due à D immitis.

mélsarsomine / RM 340 / thiacétarsamine / D immitis / prévention / traitement

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INTRODUCTION

Calvert (1987) described the situation of the American practitioners with regard to heartworm disease in dogs (HWD) as fol-

lows: "as HW infection in dogs has become more widespread, the collective awareness of the problem by dog owners has increased. Improved client education, effective screening for infection, newer diagnostic techniques, and improved treatments have evolved in recent years to make HWD treatable and preventable. If diagnosis and treatment come early enough in the course of disease, a practitioner can expect a therapeutic success rate that approaches 100% with manageable complications...".

HWD control is not so easy in practice. There is only one drug authorized in the United States for the treatment of canine dirofilariosis: a trivalent arsenical for strict intravenous injection, thiacetarsamide: TCAs (caparsolate, filaramide...). The saga of TCAs is worth recounting briefly (Courtney *et al*, 1986). In 1947, the drug was recommended at 2.2 mg/kg per day for 11 to 15 days. It was only in 1958 that a lighter protocol was tried, with a dosage of 4.4 mg/kg per day for 2 days. However, shock was common and the treatment was considered to be very risky. In 1963, an eminent practitioner, Jackson of Saint Augustine (Florida), demonstrated by means of controlled trials and critical studies completed in his practice that if this dose was divided into two daily injections, TCAs were effective and well tolerated (Jackson, 1963). Since 1963, the standard dosage is 2.2 mg/kg twice a day for 2 days, *ie*, a total of 4 injections over 48 h by strict iv. This

has been adopted and confirmed by the influential American Heartworm Society. The drug remains difficult to handle. It should only be given iv since injection by any other route (or perivascular leakage) leads to severe tissue necrosis at the injection site (Courtney, 1988).

There are few examples in veterinary medicine of trial and error procedures pursued for over 20 years by vanguard practitioners in order to obtain an essential drug because it is the only one available in its category. These generations of veterinarians can handle the drug and its constraints well, and it may seem foolhardy to attempt to develop a new injectable arsenical on the same therapeutic target HWD.

Figure 1 describes the rationale for the treatment of HWD using TCAs in the range of clinical situations which may be encountered. Accurate identification of clinical categories determines: i) whether the specific therapeutic is applied immediately or after some delay; ii) the degree of rest imposed on the dog (up to caging in the most serious forms); iii) the symptomatic treatments required. As there is only one approved standard dosage of TCAs (strict iv injection 2.2 mg/kg twice a day for 2 days), it is mainly used when HWD is moderate (class II). The practitioners in USA, Japan, Italy or Australia try to avoid the TCAs treatment when the HWD is subclinical or when it is severe or very severe.

It is understood that the control of HWD and of its adverse consequences requires specialists in internal medicine. The disease is complex and the severe cardiopulmonary effects have adverse consequence for all equilibria, excretory and satellite organs; in addition, TCAs have a narrow safety margin and treatment must be given with caution and professionalism.

In this review results of melarsomine (RM 340) developments compared to thiacetarsamide are presented.

THE PRODUCTS (FIG 2)

Thiacetarsamide: Caparsolate Ceva (USA) or Filaramide Fromm-Solvay (Italy) is used as a reference. It is supplied in 50-ml multiple dose. Each ml of 1% solution contains 10 mg sodium thiacetarsamide in isotonic solution. It is administered intravenously at 0.1 ml per pound in USA (or 0.22 ml/kg in countries using the metric system, *ie* a dose of 2.2 mg/kg) twice a day for 2 days. Thiacetarsamide is an adulticide with a molecular weight of 421.3 (% arsenic = 17.78). For 8.8 mg/kg injected the arsenic amount is 1.56 mg/kg.

The experimental product is from Rhône Merieux (France). The international non-proprietary name is melarsomine dihydrochloride, the code number: RM 340, and the registered commercial name Immiticide. It is a lyophilised white powder which gives a clear solution in distilled water, very easy to reconstitute and to inject. It is administered by deep intramuscular injection into the lumbar muscles (*longissimus dorsi*). This site was selected as the best possible for im injections in the dog (Autefage *et al*, 1990) with any drugs. RM 340 is a larvicide and adulticide with a molecular weight of 501.3 (% arsenic = 14.95). For the 2.2 mg/kg twice, 3 h apart treatment, 4.4 mg/kg are injected *ie* 0.66 mg/kg of arsenic. For the 2.5 mg/kg twice, 24 h apart treatment, 5.0 mg/kg are injected *ie* 0.75 mg/kg of arsenic. With the same target RM 340 is as good an adulticide, if not better, as TCAs using two times less arsenic.

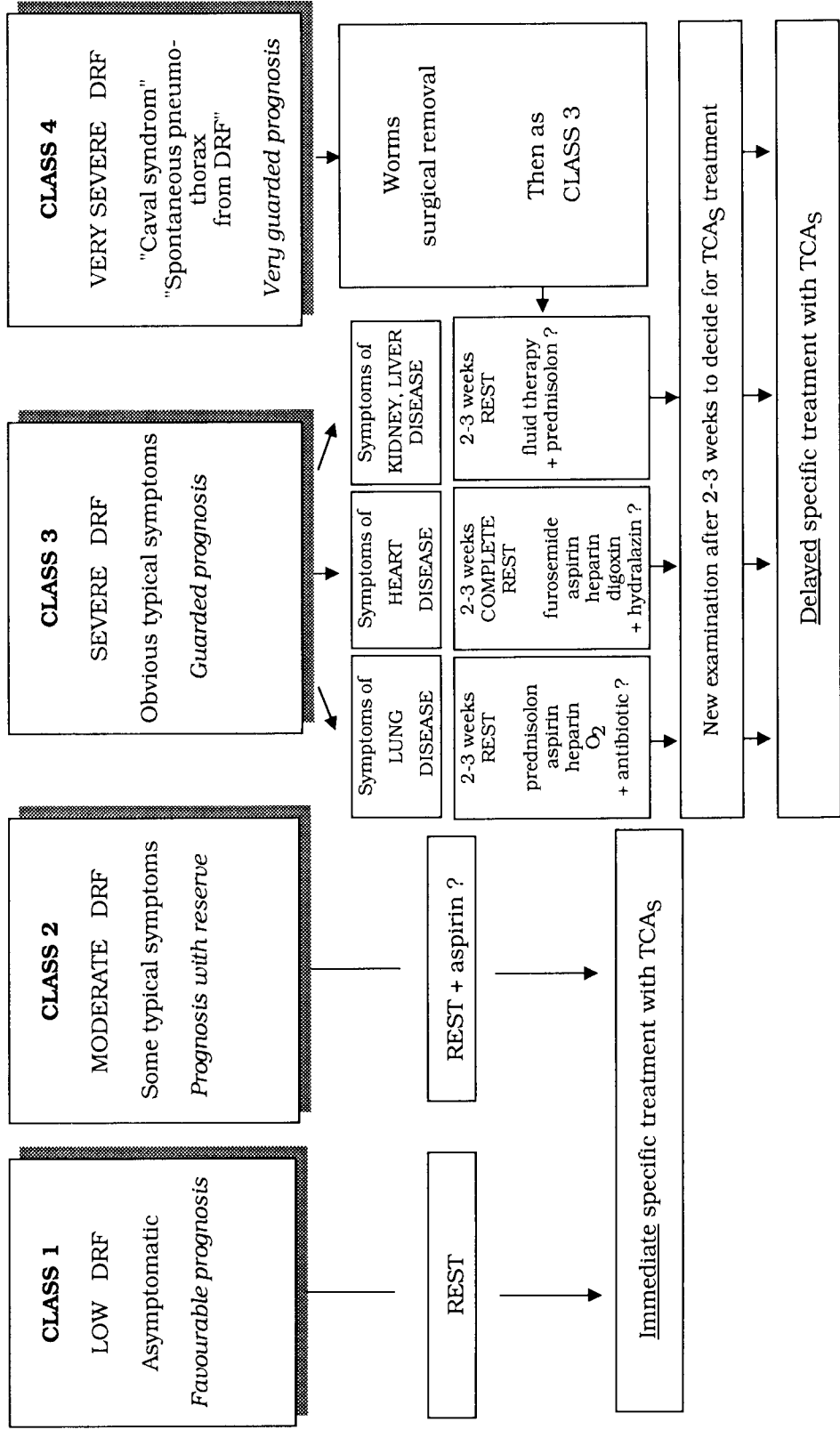
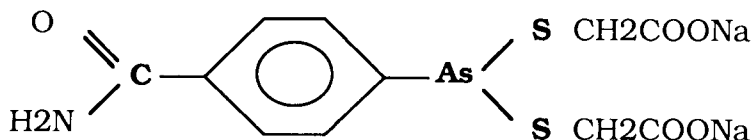


Fig 1. Rationale for the treatment of canine dirofilariasis (DRF) using thiactarsamide 2.2 mg/kg twice a day for 2 days in the various clinical classes (modified by JP Raynaud from A Vezzoni, 1988)

Family CARBAMOYL THIO ARSENITES

Substance = Thiacetarsamide disodium salt (A)

or Arsenamide disodium salt



Family MELAMINYL THIO ARSENITES

Substance = Melarsomine dihydrochloride (A) + (L)

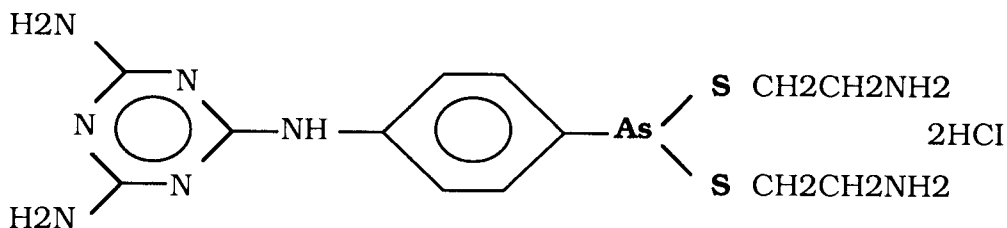


Fig 2. Formulae of thiacetarsamide or melarsomine. Macrofilaricides (L: larvicides and/or A: adulticides) to treat *Dirofilaria immitis* infections in dogs.

PHARMACOKINETICS AND PHARMACOLOGY

Arsenic as a marker

The mechanism of action for injectable trivalent arsenicals as filaricides has been described (Subrahmanyam, 1987): i) they affect glucose uptake and metabolism; ii) they inhibit the glutathione reductase; iii) they al-

ter the structure and function of the surface of the intestinal epithelium of the parasites.

Working on filariasis, Fukushima *et al* (1968) showed that it is arsenic of NaAsO_2 which inhibits SH enzymes and oxidative phosphorylation. Matsuda *et al* (1967) identified arsenic from melarsonyl dipotassium salt (Trimelarsan) as the active part of the molecule with filaricidal properties. This product from the same family as melarsomine dihydrochloride (RM 340)

has a very close chemical formula, the only difference being the lateral chains on the same nucleus: melarsenoxide. In *in vitro* cultures 5.7 $\mu\text{g/ml}$ of As are necessary for the complete killing of microfilariae. On adults, 1.7 to 5.7 $\mu\text{g/ml}$ of As reduce the production of microfilariae and 170 $\mu\text{g/ml}$ is the lethal level which accumulates in the filaria body.

More recently Holmes *et al* (1986 a, b, c) and Knight (1987) summarized this point: arsenic seems to be the active ingredient but the active chemical configuration is unknown. In an experiment 14 days after sub-therapeutic level of thiacetarsamide treatment (80% of the recommended level) the worms are still alive with an amount of 0.57 ± 0.59 or 1.63 ± 0.47 of arsenic in $\mu\text{g/g}$ of worms. The lethal level accumulated in the worm should then be higher.

To conclude, arsenic is a good marker of arsenical drug activity. Its concentration in the post-distribution phase parallels the adulticide effect.

Pharmacokinetic studies in dogs

To select optimum dosage regimen the following steps were covered and presented (Toutain and Raynaud, 1988): i) a dose response curve for efficacy was established using canine models (Dzimiński *et al*, 1989); ii) a relevant data base was implemented to modelize As disposition following RM 340 administration and to estimate the corresponding parameters; iii) linearity (dose independence) and stationarity (time independence) of arsenic kinetics were assessed to simulate any dosage regimens; iv) relationship between plasma concentration profiles and filaricidal efficacy were determined and possible effective As concentrations were qualified; v) by simulation, different effective dosage regi-

mens were generated to select the most convenient (short intervals of administration) effective and safe therapeutic programs.

Blood/plasma concentration ratio

Blood/plasma concentration in dogs was checked to select the most appropriate biological fluid for kinetic investigations. Some arsenicals (including thiacetarsamide) are extensively bound to red blood cells but adult heartworms are not hematophagous and their feeding is accomplished through the plasma. With RM 340, the blood/plasma ratio does not indicate an accumulation of As in erythrocytes even after a delay of 6 h post-treatment. Consequently, in contrast with thiacetarsamide where blood was, and is still, used for pharmacokinetic studies, plasma was selected for investigations concerning RM 340.

Pharmacokinetic parameters

The pharmacokinetic parameters after iv administration in dogs of RM 340 or thiacetarsamide (TCAs) were calculated: the most striking differences (table I) between the two products were the mean residence time which is about 5 times longer and the body clearance which is three times lower for RM 340 vs TCAs. Arsenic from RM 340 is relatively slowly eliminated.

The pharmacokinetic parameters after im administration of RM 340 in dogs were also calculated: the values were obtained using 2 im injections, either 2.2 mg/kg 3 h apart, or 2.5 mg/kg 24 h apart. The results were similar: linearity (dose independence) and stationarity (time independence) were proven.

The mean values of kinetic parameters for arsenic after 2.5 mg/kg of RM 340 ad-

Table I. Parameters of arsenic after iv injection of RM 340 or TCAs. One injection iv/mg per kg per dog (Mean \pm SD; $n = 5$ beagle dogs).

	RM 340 (plasma) 2.5 mg/kg	TCAs (blood) 2.2 mg/kg
Body clearance (l/kg per h)	0.161 \pm 0.039	0.541 \pm 0.257
Volume of distribution (l/kg)	0.73 \pm 0.13	2.2 \pm 0.93
Terminal half time (min)	180.6 \pm 57.7	177.0 \pm 106
Mean residence times (min) ^a		
– central compartment	59.9 \pm 28.2	35.9 \pm 15.3
– peripheral compartment	360.2 \pm 131.8	36.3 \pm 3.1
– total	420.1 \pm 136.2	75.6 \pm 16.0

^a From 0 to 24 h post-injection.

ministered im in dogs (mixed breeds) for 6 h (bicompartamental model) are presented in table II.

The following conclusions on bioavailability of RM 340 injected im were shown: i) the half time of absorption is very short (less than 5 min) allowing obtention of a maximal concentration in 10.7 min which makes the im injection of RM 340 very quickly and highly present in the blood, at the targeted parasite level; ii) the mean residence time (after one injection) was 153.3 \pm 113 min *ie* two to three times longer than that of thiacetarsamide; iii) there is no significant difference in relation to weight or age of the dogs.

Pharmacokinetic simulated data: the therapeutic windows

RM 340 kinetics was demonstrated to be linear and time-independent. Arsenic plasma concentration profiles corresponding to different dosage regimens (dose, interval)

Table II. Parameters of arsenic after im injection of RM 340 (2.5 mg/kg) in dogs for 6 h (bicompartamental model).

Parameters (units)	Mean \pm SD ($n = 12$)
A ($\mu\text{g/ml}$)	1.24 \pm 1.41
B ($\mu\text{g/ml}$)	0.159 \pm 0.0699
α (min^{-1})	0.0485 \pm 0.0197
β (min^{-1})	0.00183 \pm 0.00112
K_a (min^{-1})	0.230 \pm 0.097
MRT (min^{-1})	153.3 \pm 113.1
C_{max} ($\mu\text{g/ml}$)	0.59 \pm 0.16
T_{max} (min^{-1})	10.7 \pm 3.8
AUC (0–6 h) ($\mu\text{g}\cdot\text{min}\cdot\text{ml}^{-1}$)	59.8 \pm 13.1

A, extrapolated zero time plasma drug concentration of the distribution phase; B, extrapolated zero time plasma drug concentration of the elimination phase; α , slope of the distribution phase; β , slope of the elimination phase; K_a , apparent first order absorption rate constant; MRT = mean residence time in the plasma; C_{max} , maximum drug concentration; T_{max} , time for the maximum drug concentration; AUC = area under the curve of arsenic levels in plasma.

were generated in order to calculate the time A_s was above a given concentration. For possible minimum effective concentrations (MEC) from 0.08 to 0.12 $\mu\text{g/ml}$, the required minimum exposition time (MET) was determined as the shortest time during which these MEC were maintained. A dosage regimen will be presumed effective if it maintains the MEC above the requested MET. A dosage regimen will be selected to balance efficacy with tolerance and practicability. The different dosage regimens, which were used in the earliest efficacy experiments on canine models (Dzimianski *et al*, 1989) were simulated to analyse the relationships between As concentration profiles and anthelmintic efficacy response. Using this approach, a range of therapeutic windows was determined. They were defined by the combination of MEC and MET, and an overall efficacy index which is 1.00 for the reference treat-

ment (2.5 mg/kg twice, 24 h apart). The relative arsenic peak levels are also given with, again, 1.00 for the same reference treatment (0.67 mg/ml) to take into account the need to minimize intolerance or toxicity reactions and maintain the safety margin (ratio of the toxic dose to the effective dose), *ie* three times for the reference treatment (table III).

Finally, Toutain and Raynaud (1988) recommended testing in clinical conditions 2.2 mg/kg twice, 3 h apart which balances efficacy and safety and could be more convenient in practice than two 2.5 mg/kg-injections, 24 h apart. The development of RM 340 was oriented on the comparison of the two treatments in experimental models and in clinical situations to determine the best use of each one.

The same authors had the opportunity to check the validity and efficacy of the im-

Table III. Relative overall efficacy index and arsenic peak index for all the dosing intervals between the two injections.

	Dosing intervals (h) between the two injections					
	2	3	4	5	6	24
<i>Overall efficacy index</i>						
Actual exposure time/required exposure time						
Treatments						
2.5 mg/kg x 2 times	–	–	–	1.40	1.35	1.00
2.2 mg/kg x 2 times	1.23	1.22	1.19	1.13	1.09	–
2.0 mg/kg x 2 times	< 1	1.06	< 1	< 1	–	–
<i>Arsenic peak index</i>						
Actual highest peak/reference peak <i>ie</i> 0.67 mg/ml for 2.5 x 2 24 h						
Treatments						
2.5 mg/kg x 2 times	–	–	–	1.11	1.09	1.00
2.2 mg/kg x 2 times	1.03	1.00	0.99	0.97	0.96	–
2.0 mg/kg x 2 times	0.94	0.93	0.90	0.88	0.88	–

plemented pharmacokinetic model to select other posologies and therapeutic programs. The parameters being values of time above MEC in min and average concentration above MEC in $\mu\text{g/ml}$, the anthelmintic efficacy in dog models was equivalent. The results showed the power and the accuracy of monitoring pharmacokinetic values to select therapeutic programs for clinical trials.

TOXICOLOGY

Thiacetarsamide (Tcas)

After 40 years of use of this product an update has been given by Courtney (1988). On local tolerance, the drug is highly irritant and produces necrosis, but the problems are generally overcome by strict and prudent iv injection. For general toxicity, TCAs has no safety margin. Toxic reactions requiring the suspension of the treatment may occur in up to 4% of the dogs treated. The drug is known as hepatotoxic and it could also be nephrotoxic. At 50% increase in dose (3.3 mg/kg 4 times instead of 2.2 mg/kg) hepatotoxic reactions and deaths were seen in two of 11 dogs.

Melarsomine (RM 340)

The product is neither hepatotoxic, nephrotoxic nor mutagenic. The local reactions are absent in the majority *ie*: 75 to 80% of dogs used in various conditions. Mild reactions occur for 1 day in 15–20% and moderate in few dogs only (pain and/or oedema for 2–5 days).

General toxicity

RM 340 has a reasonable safety margin. For sub-clinical (class 1) or moderate (class 2) heartworm infections, the safety margin is ≥ 2 for the program 2.2 mg/kg twice, 3 h apart, and ≥ 3 for the program 2.5 mg/kg twice, 24 h apart. For severe disease (class 3) the first injection 2.5 mg/kg given for a partial killing of the worms allowing a clinical improvement of the animal, has the same safety margin of ≥ 3 .

The only symptoms generally found with the recommended programs are 1 to 2 days of anorexia, an exceptionally found pain at the time of injection and very few individuals with agitation, salivation and tremors. They are benign but the owner has to be informed of these.

When given at two times, the 2.2 mg/kg, 3 h apart treatment *ie* 4.4 mg/kg twice, 3 h apart to non-infected pound sourced dogs, Atwell *et al* (1989) showed easily diagnosed toxic signs (distress, salivation, tachycardia, tachypnoea, pawing, signs of abdominal pain, restlessness, glazed corneas, apprehension, guarded abdomen, weakness in hind legs, difficulty rising and reclining, moist crackles). The state of all dogs suffering from early toxicity was reversed with British Anti Lewisite (BAL) 3 mg/kg im. However, the use of BAL may reduce adulticidal efficacy. In case of mistake or accident BAL is the recommended antidote.

In addition to the toxicity experiments on healthy dogs, the side-effects and post-adulticide problems (thromboembolisms as an example) due to the worm lysis were also carefully controlled during clinical trials. If the drug is used with the usual rec-

ommended precautions the safety margin is maintained. Compared to TCAs, RM 340 was considered by the experienced practitioners as safer to use on infected dogs.

EFFICACY IN EXPERIMENTALLY INFECTED DOG MODELS

The experimental model

The canine models routinely used in the laboratory on beagle dogs in Athens, GA (USA) utilize infections established either by: i) iv transplantation of known numbers of worms of specified sex (generally 10 pairs = 20 worms) and age (the mature adults could be young, 7 to 12 months old, or old, 24 months old) (Rawlings and McCall, 1985); ii) subcutaneous inoculation of known number of infective larvae (generally 50 L3) from mosquitoes. The infection was used to test efficacy of the products on L5 (4-month-old parasites).

Controlled anthelmintic test (Moskey and Harwood, 1941)

The worms remaining in the controls are regarded as the probable number of worms that would have remained without treatment, while those remaining in the treated groups are worms unaffected by the compound. Percentage efficacy = $\frac{\text{mean number of worms in controls} - \text{mean number of worms in treated animals}}{\text{mean number of worms in controls}} \times 100$

When fragments were found, they could be transformed into "one dead female worm for each 25 cm in length counted..." "Non-motile intact worms are placed in warm (37 °C) physiological saline solution for 30 min and reexamined for motility. The sex of both intact live and dead

worms from each dog is recorded" (McCall *et al*, 1980; Atwell and Searle, 1989).

Overall assessment of efficacy

For impartial comparison of drug efficacy Genchi *et al* (1989) and Dzimianski *et al* (1989) proposed a new scheme for overall assessment of efficacy (OAE) of filaricidal drugs, based on a combination of the percentage of heartworms killed and the percentage of dogs cleared of worms, with specified ranges in percentages for very high, high, average, low or nil. For each treatment the OAE is given as either 'highly effective' 'partially effective' or 'marginally to non-effective'. The results obtained are judged according to those categories (table IV).

Efficacy in experimentally infected dogs

On the canine models routinely used in their laboratory (Athens, GA, USA), Dzimianski *et al* (1989, 1990) combined results of experiments are presented in table V.

The RM 340 injected once at 2.5 mg/kg is only 'partially and marginally effective' on L5 and adults, respectively. But the purpose of this single injection is to eliminate a sizeable part of the worm population (56–82%) allowing a clinical improvement in severely affected dogs (clinical class 3) when they will not tolerate the full treatment and the post-therapeutic consequences.

The two treatments 2.2 mg/kg twice, 3 h apart or 2.5 mg/kg twice, 24 h apart, are 'highly effective' on L5 or young adults, 7 to 12 months old.

In contrast (Blair *et al*, 1983) the reference product thiacetarsamide at the standard recommended regimen (2.2 mg/kg

Table IV. System used in ranking for overall assessment of efficacy based on percentage of dogs cleared of worms and number of remaining worms.

Efficacy	Overall assessment of efficacy				
	Marginally to non-effective		Partially effective	Highly effective	
	Nil	Low	Average	High	Very high
Worms killed (%)	0 – 29	30 – 69	70 – 89	90 – 96	97 – 100
Dogs cleared with residual worm number ^a (%)					
High	0 – 29	30 – 49	50 – 69	70 – 89	90 – 100
Low	0 – 19	20 – 39	40 – 59	60 – 79	80 – 100

^a High: 5 female worms or more remaining in one or more dogs or an average of 2 or more worms remaining/dog. Low: less than 5 female worms remaining in one or more dogs or an average of less than 2 worms remaining/dog.

Table V. Melarsomine (RM 340) vs thiacetarsamide (TCAs). Efficacy: overall results in experimentally infected dogs.

D immitis Stages	Immature L5 4-months old			Mature adults		
	% Worms killed	% Dogs cleared	OAE	% Worms killed	% Dogs cleared	OAE
	<i>young adults = 7–12 months old</i>					
<i>Melarsomine</i>						
2.5 mg/kg x 2/24 h apart: average (range if ≥ 2 experiments)	95.8	50.0	High eff	98.5 (96.4 – 100)	73.3 (50.0 – 100)	High eff
2.2 mg/kg x 2/3 h apart: average (range if ≥ 2 experiments)	99.3	83.3	High eff	96.0 (92.0 – 100)	75.0 (50.0 – 100)	High eff
2.5 mg/kg once: average (range if ≥ 2 experiments)	82.1	0	Part eff	55.6 (45.9 – 65.2)	10.0 (0 – 16.7)	Marg eff
<i>Thiacetarsamide</i>						
2.2 mg/kg twice a day for 2 days: (range if ≥ 2 experiments)	29.8	0	Non eff	60.2 (44.2 – 75.0)	8.3 (0 – 20.0)	Marg eff
				Old adults = 24 months old		
				85.8	25.0	Part eff

OAE = overall assessment of efficacy. High eff = highly effective. Part eff = partially effective. Non/Marg eff = non-effective / marginally effective

twice a day for 2 days) is 'non effective' in L5 or 'marginally effective' in young adults, and 'partially effective' in old adults, 24 months old.

EFFICACY IN NATURALLY INFECTED DOGS: CRITICAL TRIALS

Critical anthelmintic test (Hall and Foster, 1918)

Each animal acts as its own control and untreated controls are unnecessary.

Percentage efficacy = number of worms expelled, voided or destroyed x 100 / total number of worms for each dog. For the debris and fragments found, same as above.

The selected dogs were infected by *D immitis* as shown by parasitological examination: microfilariae positive and/or adult metabolic antigens (ELISA) semi-quantitative test, and eventually some symptoms and lesions seen by X-ray examination, echocardiography, etc...

After treatment the animals were kept in cages and necropsy was performed about 4 weeks after treatment. To count the dead and live worms and to try to reconstitute the existing population at time of treatment, the debris and fragments in the small pulmonary arteries were measured and counted for an equivalent number of dead worms: 25 cm of debris was counted as 1 female (McCall *et al*, 1980, Atwell, 1988).

When the sacrifice was earlier than 4 weeks post-treatment, too much debris are present and some worms were found alive but they would die later on. If the sacrifice was later, the worms and debris disappeared and we were then unable to give an estimate of the worm population, or a value of antiparasitic efficacy.

Results on efficacy in naturally infected dogs by critical trials

The first results obtained on efficacy on naturally infected dogs, critical tests, were published by Genchi *et al* (1989), Atwell and Searle (1989) Raynaud and McCall (1990) and included in Raynaud (1990). In the experiments 33 dogs were controlled at 2.5 mg/kg twice, 24 h apart, 42 at 2.2 mg/kg twice, 3 h apart. In table VI the individual results are allocated in categories: level of infection as worm number (low-average/high or very high). The results of the two treatments with RM 340 do not differ significantly even with a large worm population. With a low population of worms, they have at least the same level of efficacy. From these results we consider that RM 340 is a very effective drug to be used on large as well as on small populations of worms (with of course the well known precautions to control post adulticide complications when large numbers of worms are present).

In contrast, thiacetarsamide from two sets of critical tests published in the USA (McCall *et al*, 1980) with dogs from Georgia (Todd *et al*, 1980) with dogs from Illinois and (Palumbo *et al*, 1980) from Hawaii or (Courtney *et al*, 1986) from Florida showed 'partially effective' results even if the averages of 4 dogs from Hawaii with 40, 43, 141 or 210 worms are excluded.

EFFICACY IN NATURALLY INFECTED DOGS: CLINICAL TRIALS

Methods for clinical trials

The so-called 'client's dogs' are those examined and treated by a veterinary practitioner in his clinic. The following steps are taken (Raynaud *et al*, 1991; Raynaud,

Table VI. Melarsomine (RM 340) and thiacetarsamide: efficacy in naturally infected dogs. Critical trial results presented by level of infection classes.

	Level of infection (number of worms/dog)			Overall
	Low (0 – 9)	Average – high (10 – 39)	Very high (> 40)	
% worms killed % Dogs cured (dogs No)				
<i>Melarsomine (RM 340)</i>				
2.2 mg/kg x 2/3 h apart	100 100 (12)	95.6 80.8 (26)	94.4 50.0 (4)	95.7 83.3 (42)
2.5 mg/kg x 2/24 h apart	95.2 81.3 (16)	98.2 80.0 (10)	92.5 71.4 (7)	94.2 78.8 (33)
<i>Thiacetarsamide</i>				
2.2 mg/kg x times for 48 h ^a	70.2 53.3 (15)	53.9 30.8 (13)	43.1 0 (4)	58.3 ^b 42.9 (28)
2.2 mg/kg x 4 times for 48 h ^c	– –	– –	– –	72.9 37.5 (16)

^a McCall *et al*, 1980 (Georgia); Palumbo *et al*, 1980 (Hawaii). Todd *et al*, 1980 (Illinois). ^b Without the 4 very highly infected dogs from Hawai. ^c Courtney *et al* 1986 (Florida).

1991) (fig 3): i) minimum data base, to decide if the animal is kept in the experiment including: history; parasitological examination (microfilarial and ELISA test for adult antigen, semi-quantitative results); clinical examination.

When the result is negative, the dog can be put on a prevention program if he resides in an endemic area. ii) When results are positive, radiological examination and laboratory examination (blood chemistry and urinalysis) to identify class 1 dogs (sub-clinical disease) treated according to prescriptions given, should be done. iii) To precisely decide between class 2 (moderate disease) or class 3 (severe disease) and use the recommended therapeutic program, the following results are needed: electrocardiogram; hemogram; possibly echocardiography, hemostasis evaluation and tracheal wash.

Follow-up of the treatment for clinical trials

To summarize the actions needed we propose:

– for clinical classes 1 or 2: i) pre-treatment examination; ii) examination at + 3 weeks: note immediate sequels of treatment, possible local or general reactions, renewed appetite, improvement in possible behaviour. Identify possible pulmonary allergic reaction (lysis of heartworms) at the end of the first week after treatment and thromboembolisms between 7 and 20 days post-treatment; iii) eventual microfilaricide treatment: at approximately 6 weeks after treatment if direct examination or concentration test reveals microfilariae proceed with microfilaricide treatment; iv) examination at + 12 weeks: as on D0, the clinical

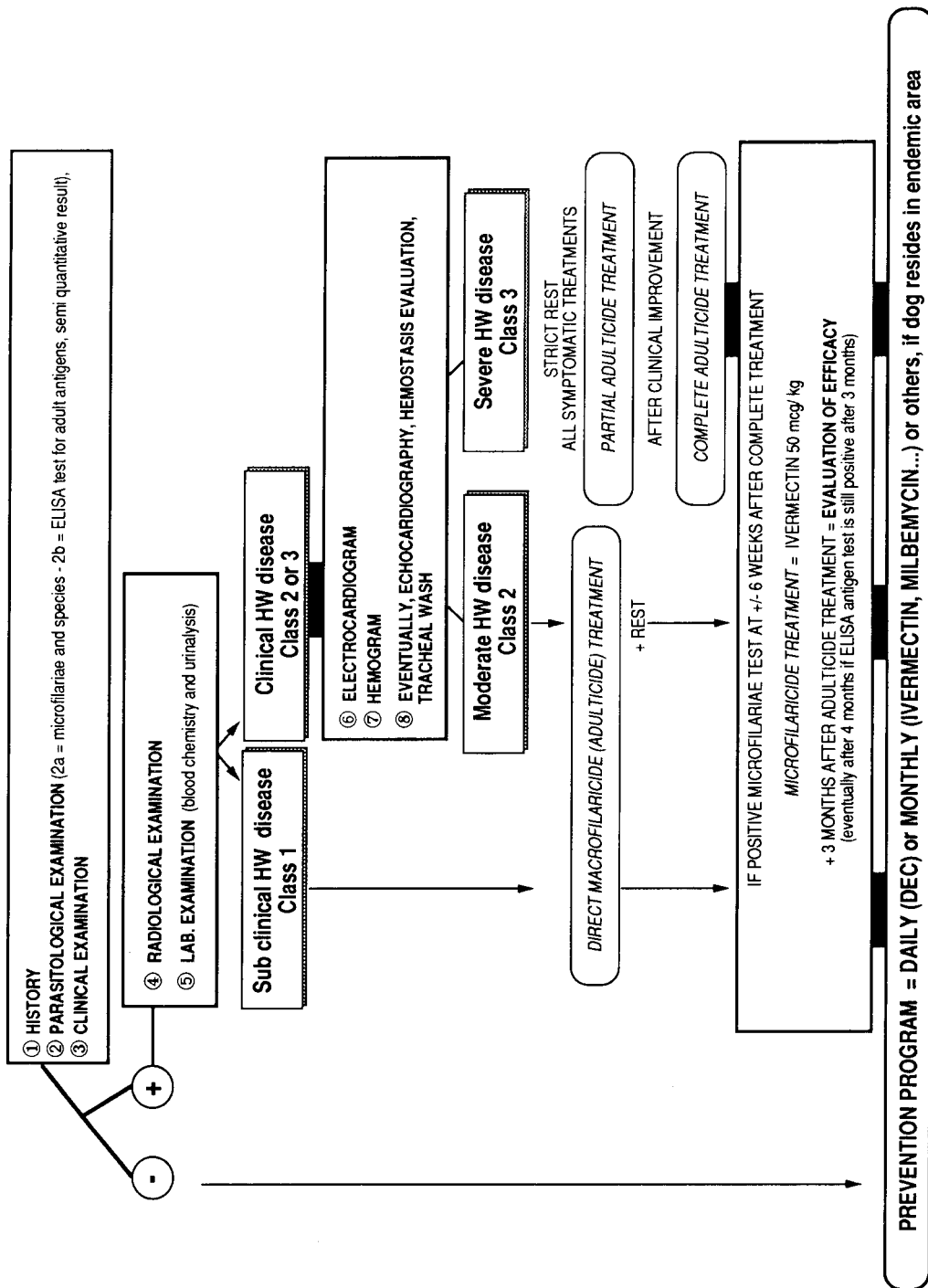


Fig 3. Diagnosis of an infection with *D. immitis* and protocol for treatment with Rim 340.

chart is filled out as completely as possible, with the exception of the radiographic examination that will only be repeated upon special indication decided by the specialist. We know that improvement in the radiological picture will take place only very slowly, several months after a successful treatment. Soluble antigens of adult heartworms detected by semi-quantitative ELISA technology disappear after the 9th week and should be absent at + 12 weeks. In case this is not shown, we request a final test after 4 months. If the situation requires, begin prevention program if dog resides in an endemic area; v) examination at 4 months: only if the test for circulating antigens is still positive at + 12 weeks. Otherwise, this examination is unnecessary;

– for clinical class 3 (severe disease): i) partial RM 340 treatment: strict rest, better in cage for 15 days do all necessary symptomatic treatments and one injection of RM 340 at 2.5 mg/kg, one to 2 weeks later; ii) clinical results after one month: have the major symptoms and the general condition of the animal improved? Have essential functions been maintained, improved? a) If not, continue symptomatic treatments and rest for another 3–4 weeks, then give RM 340 treatment; b) if yes, give complete RM 340 treatment. This decision entails repeating laboratory examinations; iii) full RM 340 treatment: 2.5 mg/kg twice, 24 h apart. Along with this treatment, continue symptomatic treatments and strict rest; iv) examination at + 12 week, examination at + 4 months: apply general protocol.

Recording of local or general reactions and thromboembolisms

Local reactions

Local reactions were noted as follows: 0 (no symptom); + (slight pain and/or transient oedema); ++ (pain and/or oedema for

2–5 days); +++ (serious pain and/or oedema).

General reactions

General reactions were noted as follows: 0 (no symptom); + (transient anorexia and/or sickness ≤ 1 day), ++ (anorexia and sickness 2–7 days); +++ (serious reactions, possible death).

Thromboembolic reaction

Thromboembolic reaction was noted as follows: 0, + (slight, transient reactions, non-treated or treated with corticoids); ++ (serious reactions, death in 7 to 20 days).

Antiparasitic efficacy

Antiparasitic activity was determined as follows: On microfilariae, on adults antigens controlled by ELISA. Results (good) – (intermediate) – (no efficacy) 90 to 120 days post-treatment.

Clinical cure

Clinical cure was noted as follows: short term (during the first 2 weeks), or long-term (90 to 120 days post-treatment); 0, + (partial cure some symptoms), ++ (cure); +++ (100% cure, improvement of clinical class number).

Results of clinical trials

In veterinary practice RM 340 with its final formulation and presentation has been used in some countries, France, Australia and Italy, following the recommended therapeutic program. A summary of the overall results (Raynaud, unpublished data) is presented (table VII).

Forty percent of the dogs in the experiment were in clinical class 1, 40% in class 2 and 20% in class 3, the dogs being

Table VII. Clinical trial results (% dogs) of RM 340 treatment according to clinical classes.

	Class I	Class II	Class III
<i>Thromboembolisms</i> ^a			
0, no reaction	100	95.9	75.8
+, mild reaction ^b	0	4.1	24.2
++, death	0	0	0
<i>Antiparasitic efficacy</i>			
Total cure ^c	97.0	99.0	97.6
<i>Clinical efficacy (on symptoms)</i> ^d			
0, no change	—	0.8	0
+, reduction in severity	—	9.3	10.9
++, disappear of one of the main symptoms	—	33.9	26.1
+++, disappearance of all	—	55.9	63.0

^a Between 5–20 days post-treatment (trials on 143 dogs in Italy following the prescribed actions for class III dogs; Genchi *et al.*, 1991; Vezzoni *et al.*, 1989). ^b Resolved spontaneously or after short-term corticoid therapy. ^c Disappearance of adult worm antigens within 12 weeks of treatment (ELISA test). ^d At 84–90 days post-treatment. Disappear: disappearance.

young (≤ 3 years) for 28.7% and old (≥ 9 years) for 13.5%.

Local and general tolerance

The product was responsible for mild reactions (++) on local tolerance in 7.8% of the dogs and general reactions in 20.4% of the dogs. No dog showed severe reaction and the product is thus considered by practitioners as safe under their conditions of use.

Post-treatment thromboembolisms

This accident is well identified as 'post-adulticide effect' after thiacetarsamide treatment (Calvert, 1987; Knight, 1987; Courtney, 1988; Vezzoni and Genchi, 1989). From results obtained in field practice (table VII) in Italy with specialized practitioners (Vezzoni *et al.*, 1989; Genchi

et al., 1991) we consider that RM 340, even if very effective, does not present thromboembolism risk at a significant level.

Antiparasitic effect (table VII)

As judged by the disappearance of adult worm antigens within 12 weeks of treatment (ELISA test), the RM 340 treatment is very effective in practice with an overall result of 98.0% which can be considered as very good.

Clinical efficacy (table VII)

For animals with symptoms on day 1 of treatment (class 2 or class 3 dogs), very few had no improvement 12 weeks after (0.8% in class 2 and no animal in class 3); few (about 10%) had only a reduction in the severity of symptoms, but about 56–63% has a complete cured *ie* a complete

disappearance of the symptoms, which is considered as a very good result.

For the specific case of class 3 animals, Vezzoni *et al* (1989) and Genchi *et al* (1991) have shown that a single injection of 2.5 mg/kg given in parallel with symptomatic treatment and complete rest is very beneficial. It gives a partial cure and the animals can then receive the full treatment without any risk and with full efficacy.

ASSESSMENT OF CARDIOPULMONARY FUNCTION IN RELATION TO ADULTICIDE THERAPY (PRELIMINARY RESULTS)

Chronic pulmonary hypertension during heartworm (HW) infection is associated with arterial disease and in particular myointimal proliferation (Rawlings and Tackett, 1990). Dogs treated with adulticide have dying HW that shift into the smaller arteries where they fragment and undergo phagocytosis. Accentuation of pulmonary hypertension and parenchymal disease can lead to congestive heart failure and/or acute pulmonary dysfunction in severe cases.

A study was made (Rawlings, personal communication, 1990) to determine the efficacy of RM 340 in improving the clinical status of severely and moderately affected HW infected dogs, and to compare with TCAs (thiacetarsamide) their ability to improve cardiopulmonary functions.

On 9 high and 8 low HW burden dogs treated with TCAs and 6 high and 5 low HW burden dogs treated with RM 340, pulmonary hemodynamic studies included measurements of direct and mean pulmonary arterial pressure and thermodilution measured cardiac output with calculation of cardiac index. This resulted in values for

total pulmonary vascular resistance. The author concludes that there does not appear to be any difference between the two adulticides in the severity of pulmonary thromboembolism or hypertension in the week following treatment. There is also the suggestion that the RM 340-treated dogs had a greater resolution of their disease after 3 weeks of treatment ($P < 0.05$) (fig 4).

With a different technology, Haroutunian (1990) has shown in infected dogs that bi-dimensional echocardiography and Doppler echocardiography gave useful parameters; acceleration time (ACT) and right ventricular ejection time (RVET) as a ratio (ACT/RVET) can possibly be correlated to mean pulmonary arterial pressure (PAP). The normal range being ACT/RVET 0.45–0.55, for 0.40 the PAP value is about 30 mmHg (moderate hypertension); if ACT/RVET = 0.35, PAP is 40 mmHg (hypertension value). When ACT/RVET = 0.30 or less, it corresponds to 50 mmHg, *ie* a severe to very severe hypertension. Haroutunian and Atwell (personal communication, 1990) have compared the post-adulticide effect on ACT/RVET with thiacetarsamide ($n = 5$) or RM 340, 2.2 x 2/3 h apart ($n = 10$) treatments. They conclude that there is a dramatic difference between the two drugs: on +1 +2 and +2 +3 days after treatment the difference is significant ($P < 0.01$) when RM 340 decreases the values of hypertension (ACT/RVET normal = 0.49 at +1 +2 days and 0.46 at +2+3 days) TCAs slightly increases the same hypertension (ACT/RVET = 0.36 at day + 1 and 0.31 at day 2). On day +5 +6 with RM 340 ACT/RVET is 0.43 (hypertension normal–moderate) and with TCAs ACT/RVET is 0.35 (high hypertension). The differences between the two treatments were significant ($P < 0.05$). On day +13, +14 with RM 340 ACT/RVET is 0.46 (normal) when with TCAs ACT/RVET is 0.38 (moderate hyper-

Mean pulmonary arterial
pressure/cardiac output
(mean \pm SD)

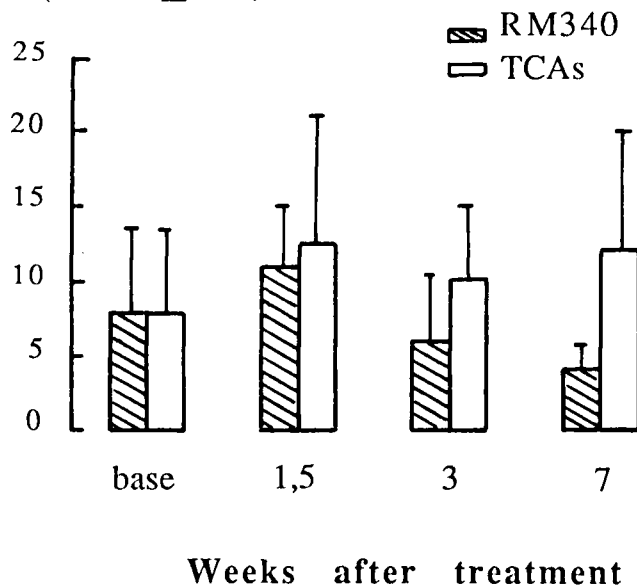


Fig 4. Total pulmonary resistance (mean pulmonary arterial pressure/cardiac output) during normoxia at base and after treatment with RM 340 or thiacetarsamide (Rawlings, 1990; personal communication).

tension), the differences being significant ($P < 0.05$) (fig 5).

Conclusion. Based on small groups of treatment dogs, there could be a difference between the effects of the two products. In contrast to RM 340, TCAs seems to increase PAP in the early days after treatment. The beneficial effects of RM 340, as suggested by these pilot trials during the days following treatment, may be contributory to the reports of obvious clinical improvement. Further studies are required to substantiate these results.

**POSSIBLE USE OF RM 340,
A TACTICAL TREATMENT
TO PREVENT HW DISEASE
(PRELIMINARY RESULTS)**

In Italy, Morocco, Japan and France, the tactical treatment to prevent HW disease was tested on naive or on infected dogs, treated twice a year with 2.2 mg/kg \times 2 injections 3 h apart, in the middle of the mosquito season (mid August) and after the end of the season (December–January). At all the sites the results obtained to prevent HW infection or disease were good to

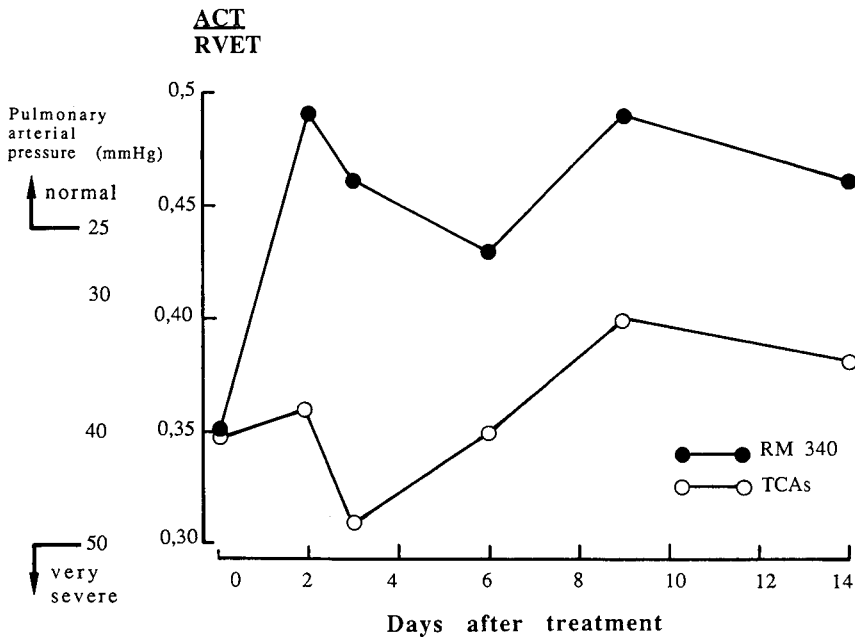


Fig 5. Doppler echocardiography on infected dogs (class 2 or class 3): acceleration time / right ventricular time (ACT/RVET) in days after treatment with thiacetarsamide (TCAs) or RM 340 (2.2 mg/kg twice, 3 h apart) (Haroutunian and Atwell, 1990; personal communication).

excellent. Results obtained in the USA after a full year of control have been presented recently (McCall *et al*, 1990 a, b; McTier *et al*, 1990).

Considering the high level of activity of RM 340 against adult and 4-month-old immatures of *D immitis*, three similar field studies were conducted in states with moderate (Georgia, Florida) or high (Louisiana) enzootic potential to determine the effectiveness of RM 340. In each study, 30 naive beagles, allocated to groups of 5 'tracer dogs' each, were placed under field conditions for various intervals from April 1988 to April 1989. Transmission occurred at all of the three sites during the periods of April to August and August to December, but no transmission was evident at

any of the three sites during the period from December to April. Based on worm measurements and microfilaremia data, most of the transmission occurred in late July–August and early September, with heavier worm burdens in Louisiana compared to those in Georgia and Florida.

Dogs on the 'tactical program' were exposed for 12 months and treated on 2 occasions, *ie* mid-mosquito season = August and after mosquito season = December. Treatments were initiated in August 1988. Each treatment consisted of 2 deep im (lumbar) injections of 2.2 mg/kg 3 h apart. At each site, one group of non-treated control dogs was exposed for 12 months and each of 3 groups of 'tracer' dogs was exposed for three consecutive 4-month peri-

ods (April, August, August–December, December–April). All dogs were bled at specified times for collection of serum and examination for microfilariae. All dogs were brought indoors at the last treatment (and/or after designated exposure) and held for 5 months before necropsy. All of the dogs on the 'tactical program' were free of heartworms, whereas most of the 'tracer' non-treated dogs exposed for an 8-month period (April–August, August–December) at the three sites had worms and while worms were recovered from all of the control (non-treated dogs exposed during 12 months–April 1988 to March 1989—in Georgia (average: 6.75, range/dog: 5–8) and Florida (average: 5.4, range: 1–13) and from 4 of the 5 in Louisiana (average: 25.2, range: 0 to 45).

Follow-up studies to confirm the unexpected and surprising lack of transmission from December to April are underway. Others experiments are in progress with long term control of infected dogs microfilariae and adult antigens positive at start.

These results are promising for a possible twice-a-year treatment to control heartworm disease.

CONCLUSION

RM 340 is effective and safe in the majority of conditions seen; in practice a strict protocol for use has to be followed. It is summarized here:

Clinical classes

It is essential to determine the severity of the HWD. The dog must be assigned to one of the four predefined clinical classes (class 1: subclinical; class 2: moderately severe disease; class 3: severe disease;

and class 4: very severe disease) (Raynaud *et al*, 1991). i) To decide whether the dog should be put directly onto a chemoprophylaxis program; ii) to determine the prognosis of the disease; iii) to establish treatment: to adapt the therapeutic program to each clinical class, to decide upon conditions of treatment (strict rest or not), the organize the necessary symptomatic treatment and preventive treatment for thromboembolism, to objectively evaluate clinical improvement following treatment.

Weighing up of clinical classes for application of therapeutic programs

A schematic description of the defined clinical classes makes it possible to situate animals representative of these classes. However, before actually applying the treatment, weighing up factors in the form of nuances and precisions must be introduced. As a precautionary measure in conducting treatments, the clinical class must be over-estimated as follows:

Dogs in class 1 that should be treated as class 2 dogs

Very small dogs, older dogs (more than 9–10 years), heavily infested animals (semi-quantitative soluble adult antigen tests, ELISA methodology) with surveillance for thromboembolic risks 1 week after treatment.

Class 1 dogs that should be treated as class 3 dogs

Dogs with hepatic or renal failure (whatever the cause).

Class 2 dogs that should be treated as class 3 dogs

Dogs over 10 years of age, heavily infested animals, dogs with hepatic or renal failure.

During the development of RM 340 the aim was to define a therapeutic program adapted to each clinical class. This objective was achieved with the help of specialized practitioners involved in the clinical trials (Vezzoni *et al*, 1989; Raynaud, 1990). This gave the opportunity to both intensify and clarify the rational approach to the determination of clinical classes in dogs suffering from HWD (Raynaud, 1991; Genchi *et al*, 1991).

Directions for use of melarsomine dihydrochloride (RM 340)

Dosage choice and treatment protocols (see fig 6): HWD treatment as a function of clinical classes

Class 1 subclinical heartworm disease

The preferred treatment is 2.5 mg/kg administered twice (im injections) at a 24-h interval. However, the dosage of 2.2 mg/kg in 2 injections at 3-h interval is equivalent in both efficacy and safety. The choice is made on the basis of convenience, availability of the owner or the veterinarian. After treatment, all strenuous exercise (hunting, running) must be avoided for 2–3 weeks.

Class 2 moderate heartworm disease

The preferred treatment is 2.5 mg/kg, 2 injections at a 24-h interval. Rest is necessary after treatment, and no exercise, other than walking on a leash, should be allowed.

Class 3 severe heartworm disease

The protocol is more complex, because the disease itself is complex, often serious with lesions of several essential organs. Symptomatic supportive treatment for 1–2 weeks and strict cage rest are necessary. One injection of 2.5 mg/kg provides a partial antiparasitic efficacy of approximately 50% efficacy in worm kill that allows the

dog, placed under supportive treatments and cage rest, to better tolerate the elimination of all the parasites at the time of full treatment.

Symptomatic treatments for prevention of thromboembolism and improvement of arterial lesions to treat heart failure, pulmonary hypertension, etc... are strictly applied. The full antiparasitic treatment (2.5 mg/kg twice at a 24-h interval) is given after 30 to 60 days when the clinical picture has improved.

Precautions and warnings

The dog should be restrained correctly for the intramuscular injection in the lumbar muscles. In case an injection is partially subcutaneous or in fat (obese dog) or between muscles (frequent if the injection is given in the thigh, which is not recommended because of this), transient oedema may occur.

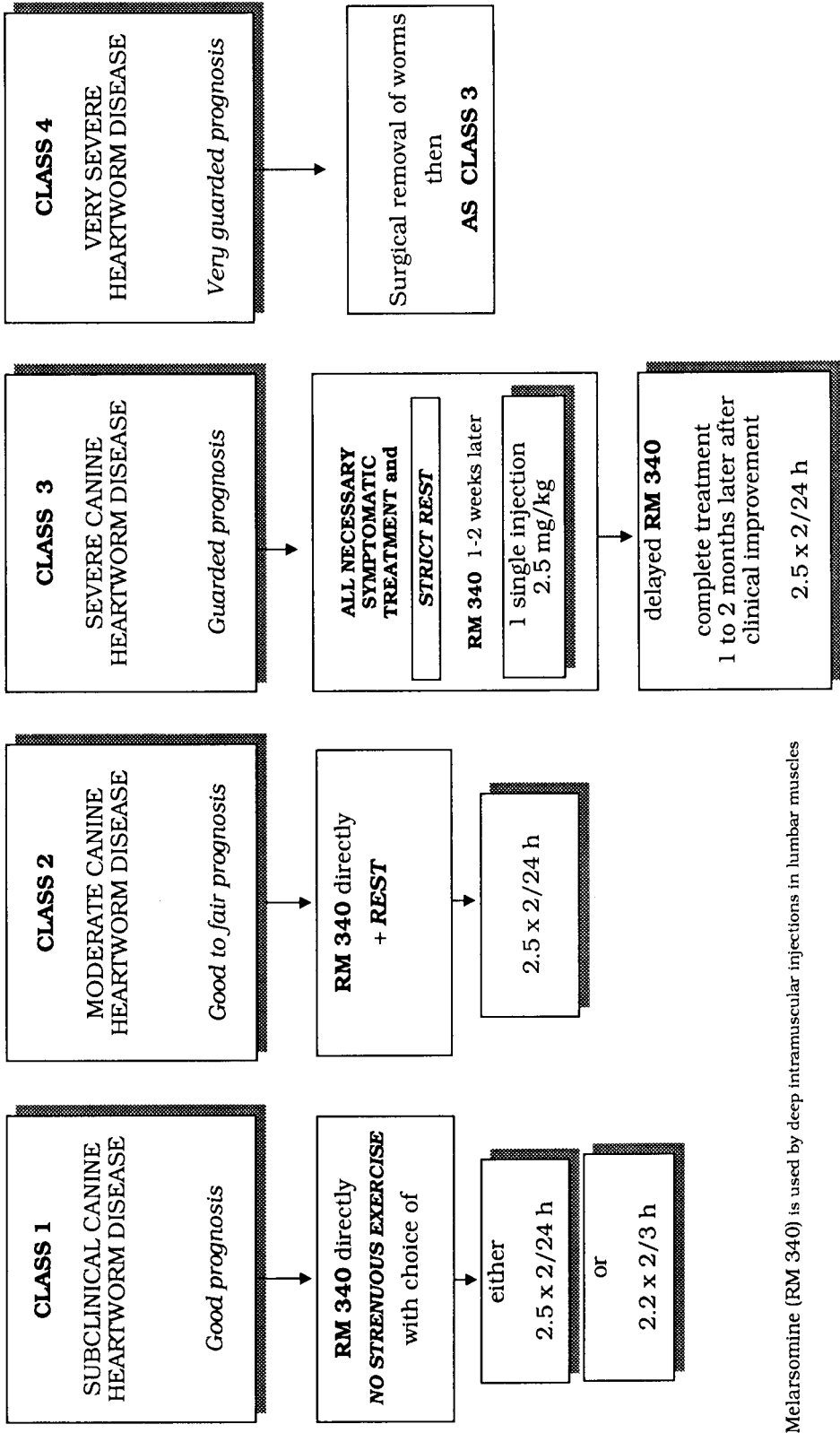
Exceptionally, a fleeting painful reaction may occur at the time of injection. On the other hand, temporary anorexia, lasting a maximum of 2 days, is frequent. The owner should be informed of this. A few animals may be agitated, with or without excessive salivation and tremor, for a brief period after the injection. The veterinarian should be aware of these symptoms; they are benign.

Antidote

It has been shown that, after a voluntary overdose, British Anti-Lewisite (BAL) at the dosage of 3 mg/kg, administered twice or three times at a 3-h interval in deep intramuscular injection, is a selective and strong antidote (Atwell *et al*, 1989).

Follow-up of treatment

No strenuous exercise (hunting, running) should be allowed for class 1 dogs for 2–3



*Melarsomine (RM 340) is used by deep intramuscular injections in lumbar muscles

Fig 6. Canine heartworm treatment with melarsomine dihydrochloride (RM 340) as a function of clinical classes. * Melarsomine (RM 340) is used by deep intramuscular injections in lumbar muscles.

weeks. Treatment for classes 2 and 3 should be followed by strict limitation of all exercise (hospitalized dog, cage rest or at home only walked on a leash. A cage is mandatory for dogs with right-sided heart failure). Dogs in class 3 require strict rest as soon as symptomatic treatments have begun. This rest is essentially necessary to limit thromboembolic effects, which are frequent when the parasitic load is heavy and/or the animal reacts strongly. The normal duration of rest is 3 weeks or more after treatment.

At the end of the first week of treatment, the owner may observe symptoms of fever, anorexia and depression that signify an allergic reaction of the lungs to foreign bodies, *ie* the first heartworms killed by the treatment that have undergone lysis. A corticosteroid treatment is very efficacious in resolving this disorder. The owner may use a prescription prepared in advance to carry out this treatment.

An expected and frequent episode of thromboembolism may occur between 7 and 20 days after treatment. The signs are fatigue, depression, anorexia, polypnea and dyspnea. This episode, which is sometimes difficult to authenticate when appropriate thromboembolism treatment has been given can be benign or severe. Only the veterinarian can organize a treatment, when necessary.

When microfilariae have been identified, by concentration techniques 30–50 days after RM 340 adulticide treatment, microfilaricide treatment is recommended. The dog should be rechecked for soluble antigens of adult heartworms with the ELISA semi-quantitative methodology, 12 weeks after adulticide treatment. We recommend using the same test for this check as the one used on D1.

Cardiac symptoms regress slowly, but a rapid improvement in clinical symptoms and

a return to dynamic behaviour and appetite are seen. Owners notice this improvement.

If the animal remains in an endemic area, prophylaxis should be carried out during the mosquito season.

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