NON-IONIC SURFACTANT VESICLE FORMULATION OF STIBOGLUCONATE FOR CANINE LEISHMANIASIS

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Leishmania infantum occurs in the Mediterranean where the dog, a reservoir of infection for man is a veterinary problem, canine visceral leishmaniasis responding poorly to therapy with the pentavalent antimonials which are also used in man. Carrier mediated therapy in the dog provides an opportunity to obtain pharmacokinetic data, which is not readily achievable in small animals, for the carrier, and free, forms of the drug but introduces the problem of scale up of the vesicle formulations. Due to the cost of the phospholipid, liposomal formulations are expensive and we describe here an inexpensive, stable, sterile formulation of sodium stibogluconate in non-ionic surfactant (NIS) vesicles for use by i.v. administration in the dog.

Vesicles comprised the NIS IV (L'Oreal), cholesterol (CHOL) and dicetyl phosphate (DCP) in a 5:4:1 molar ratio. 1.21g (3 mmol) of a melt (90-95 °C) of NIS/CHOL/DCP was hydrated by the rapid addition of 20 mL 100 mg mL^{-1} sodium stibogluconate (Welcome Foundation) and further vigorous mixing. Hydration at 70°C with agitation was continued for 2 h. The suspension was then extruded 10 times through two 50 nm polycarbonate membranes (Nucleopore), using a jacketed (70°C) extruder (Lipex Biomembranes). Free drug was removed from the vesicle suspension by gel filtration (18 x 2.6 cm Ultrogel AcA22 column using pH 7.4 Tes/His/NaCl buffer as eluant), the vesicles appearing in the void volume to yield 15 mL of suspension at a concentration of 1.25 mg Sb mL^{-1} and 5.61 mg mL^{-1} NIS/CHOL/DCP mixture, which represents an entrapment of 90 g Sb per mol vesicle forming material. Eluted suspension was immediately sterilised by filtration. Vesicles in diluted suspensions, sized by photon correlation spectroscopy (Malvern Instruments 60 channel, 7027 correlator, at a 90° scattering angle using a 10 mW He/Ne laser, Liconix, at 632.8 nm), were 127 nm in diameter. Free and vesicular forms of stibogluconate were administered to mongrel dogs (wt., 12 - 24 Kg) via the antecubital brachial vein and at various time intervals blood samples were removed from the contra-lateral vein for assay of Sb. Weighed samples of lyophilised tissue and body fluids were digested in nitric acid and the Sb content of the diluted digests determined by hydride-generation atomic absorption spectrometry. Dogs were killed at 3 (fig.1a) and 24 (Fig. 1b) h after stibogluconate administration by intravenous barbiturate overdose for the removal of various target tissues; liver (l), spleen (s), bone marrow (femur, f; sternum, st), popliteal lymph nodes (p) and non-target tissues; heart (h), kidney (k), urine (u) and blood (b) for Sb assay. At short time intervals after dosing the vesicular formulation modified blood levels such that peak levels were lower and less marked than after free drug with an associated increase in tissue loading (Fig 1a), which by 24 h post-dosing (Fig 1b), was even more apparent. No toxic effects were observed although levels in some non-target tissues (e.g.heart) were elevated.

Figure 1. Tissue Sb levels in the dog at 3 (1a) and 24 (1b) h after stibogluconate dosing.