Safety of Intravenous Administration of Acridine Orange in Dogs

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ABSTRACT

Acridine orange (AO) has been used for photodynamic therapy. In human medicine, it has been used locally after cytoreductive surgery. However, local administration of AO solution does not appear to result in penetration into deep tissues. Therefore, in invasive tumors, systemic administration of AO is useful. The purpose of this study was to evaluate the short-term safety of intravenous administration of AO (0.1 mg/kg) in dogs.

Five beagles were used in this study. Initial evaluation (control) consisted of a physical examination, complete blood count (CBC), serum chemistry, and serum AO concentration. Clinical signs were observed every day for 1 month. CBC and serum chemistry were obtained 1, 3, 7, and 30 days after AO administration. Serum AO concentrations were measured 0, 7.5, 15, 30, 60, 90, 120, 150, 180, 240, and 300 min after AO 0.1 mg/kg was administered into the cephalic vein over 30 s.

All dogs showed no clinical signs for 30 days. No photosensitivity was noted. All CBC and serum chemistry results were within normal limits. After intravenous injection of AO (0.1 mg/kg), serum AO level decreased rapidly and was below the detection limit (5 ng/mL) 2 hr after injection.

These results show that intravenous administration of AO 0.1 mg/kg was safe on a short-term basis. Systemic administration of this drug should be limited to dogs with malignant tumors and a short lifespan, because the long-term effects of systemic AO are unknown.

INTRODUCTION

Acridine orange (AO) was first extracted from coal tar as a weak basic dye over 100 years ago.[1] The photosensitizing effect of AO is well established,[2] and this drug is used in photodynamic therapy.[1, 3-6] The

success of this treatment has been reported in mouse epithelial tumors[6] and rat gastric tumors.[7]

Kusuzaki et al. (2005) advocated AO photodynamic therapy (AO-PDT).[8] In intralesional or partially marginal tumor excision, AO solution was administered locally, with removal of excess AO and washing out with saline. Then, the tumor was fluorovisualized by blue light excitation.The visualized tumor was treated surgically, and residual tumor was irradiated by xenon light to eradicate tumor cells. In an in vitro model, AO combined with low-dose X-ray irradiation of about 1 to 5 Gy had a strong cytocidal effect on cultured mouse osteosarcoma cells (radiodynamic therapy with AO, AO-RDT).[9] Kusuzaki et al. (2005) reported good local control of musculoskeletal sarcomas in humans.[8] It appears that local administration of AO solution does not result in penetration into deep tissues. Therefore, in invasive tumors, systemic administration of AO is useful for AO-RDT. Satonaka et al (2010) reported anti-tumor activity with intravenous (IV) administration of AO 1 mg/kg, followed by illumination in a mouse osteosarcoma model.[10] However, the toxicity of systemic AO has not been well studied.

Satonaka et al (2006) reported that, based on the results of an acute toxicity study of AO, the estimated LD_{50} of this substance following IV administration was 27.30 mg/kg in mice.[11] However, the safety of AO in dogs has not been confirmed. The purpose of this study was to evaluate the short-term safety of IV administration of AO (0.1 mg/kg) in dogs.

MATERIALS AND METHODS

Five male beagles were used in this study. The dogs' age ranged from 5.4 ±1.6 years (mean ± SD), with a body weight of 13.2 ± 1.6 kg. All procedures were performed in compliance with the guidelines of the Animal Research Committee of Azabu University.

Initial evaluation (control) consisted of a physical examination, complete blood count (CBC), serum chemistry, and serum AO concentration. Clinical signs were observed every day for 1 month. CBC and serum chemistry were obtained 1, 3, 7, and 30 days after AO administration. Serum AO concentrations were measured 0, 7.5, 15, 30, 60, 90, 120, 150, 180, 240, and 300 min after AO administration. The dogs were kept under fluorescent light with no light shielding.

AO (Acridine orange hydrochloride solution, 10 mg/mL in H_2O, Sigma-Aldrich, St. Louis, MO, USA) 0.1 mg/kg was administered into the cephalic vein over 30 s.

Determination of Serum AO Concentrations

Serum AO concentrations were determined by high-performance liquid chromatography with a fluorescence detector (Ex 492 nm, Em 523 nm). Separation was achieved with a 3.0 mm × 75-mm column (Ascentis Express, Sigma-Aldrich) in which temperature was maintained at 40°C. The mobile phase composition was 0.025% phosphoric acid and 0.1% octanesulfonic acid in water as mobile phase A, and 0.025% phosphoric acid and 0.1% octanesulfonic acid in 80% acetonitrile (20% water) as mobile phase B. A linear time gradient program [Time (min)/%B: 0/30, 3/42, 5/44, 5.01/100, 8/100, 8.01/30] at a flow rate of 1.0 mL/min was used. A mixture of serum sample (400 µL) and acetonitrile (800 µL) was centrifuged at 15,000 g for 10 min at 4°C. The supernatant of the mixture (5 µL) was injected and chromatographed under the above conditions. Each sample was measured in duplicate, and a standard curve was prepared in normal dog serum. The limit of quantification of the method was 5 ng/mL. The method was linear between 5 and 100 ng/mL. Inter- and intra-assay coefficients of variation were <10%.

Statistical Analysis

The parameters of CBC and serum chemistry were compared using one-way analysis of variance for repeated measures and a posteriori testing with Dunnett's multiple comparison test. Differences were considered significant at $p < 0.05$.

***Table 1**. CBC and serum chemistry results over time*

	Reference range	Control	1 day after administration	3 days after administration	7 days after administration	1 month after administration
Total WBC ($\times 10^3/\mu L$)	6-17	10.8 ± 2.3	13.0 ± 3.8	12.5 ± 1.6	13.0 ± 3.2	13.8 ± 5.0
RBC ($\times 10^6/\mu L$)	5.5-8.5	6.62 ± 0.58	6.13 ± 0.94	6.78 ± 0.35	6.60 ± 0.31	6.61 ± 0.54
Hemoglobin (g/dL)	12-18	15.7 ± 0.7	14.7 ± 2.3	15.9 ± 0.6	15.6 ± 0.8	15.4 ± 1.5
PCV (%)	37-55	45.4 ± 2.5	42.1 ± 6.2	46.2 ± 1.2	44.9 ± 1.6	45.0 ± 4.0
MCV (fl)	66-77	68.7 ± 3.5	68.7 ± 3.4	68.2 ± 3.2	68.0 ± 3.1	67.2 ± 1.6
MCH (pg)	18.8-24.5	23.8 ± 1.5	24.1 ± 1.7	23.5 ± 1.5	23.7 ± 1.6	23.0 ± 0.5
MCHC (%)	32-36	34.7 ± 0.9	35.0 ± 0.9	34.4 ± 0.7	34.8 ± 1.0	34.2 ± 0.3
Platelets ($\times 10^3/\mu L$)	200-500	327 ± 121	358 ± 188	379 ± 947	373 ± 98	257 ± 29
Total protein (g/dL)	5.2-8.2	6.6 ± 0.6	6.3 ± 0.5	6.6 ± 0.6	6.4 ± 0.5	6.4 ± 0.5
Albumin (g/dL)	2.7-3.8	3.0 ± 0.4	2.9 ± 0.4	3.0 ± 0.4	2.9 ± 0.3	2.9 ± 0.3
A/G		0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.3
Aspartate amino-transferase (IU/L)	0-50	30.2 ± 4.6	30.6 ± 9.4	31.4 ± 7.5	29.2 ± 6.6	30.2 ± 5.3
Alanine aminotrans-ferase (IU/L)	10-100	46.2 ± 16.4	45.0 ± 14.1	51.8 ± 18.7	46.4 ± 15.2	37.2 ± 17.0
Alkaline phosphatase (IU/L)	23-212	79.2 ± 33.7	80.8 ± 30.2	74.8 ± 27.0	69.8 ± 19.6	71.0 ± 12.6
γ-glutamyltransferase (IU/L)	0-7	4.0 ± 1.0	4.2 ± 0.8	4.8 ± 1.3	4.6 ± 0.5	4.8 ± 0.8
Amylase (IU/L)	500-1500	793 ± 177	748 ± 146	790 ± 154	779 ± 68	936 ± 67
Lipase (IU/L)	200-1800	267 ± 120	283 ± 197	275 ± 79	249 ± 79	426 ± 183
Urea nitrogen (mg/dL)	7-27	11.8 ± 1.1	10.0 ± 1.6	14.2 ± 4.7	13.8 ± 5.8	17.0 ± 5.6
Creatinine (mg/dL)	0.5-1.8	0.60 ± 0.07	0.62 ± 0.08	0.70 ± 0.12	0.62 ± 0.08	0.76 ± 0.09*
Total cholesterol (mg/dL)	110-320	146.0 ± 15.3	134.8 ± 8.5	141.0 ± 11.8	137.6 ± 8.8	138.2 ± 12.0
Triglycerides (mg/dL)	10-100	53.4 ± 46.7	21.4 ± 5.5	75.0 ± 41.2	64.8 ± 30.9	38.6 ± 14.7
Na+ (mEq/L)	134-153	148.4 ± 2.9	147.4 ± 3.2	147.0 ± 2.7	147.8 ± 1.8	147.2 ± 2.4
Cl- (mEq/L)	105-118	111 ± 2	112 ± 2	110 ± 4	111 ± 3	114 ± 2
K+ (mEq/L)	3.4-4.6	3.9 ± 0.4	4.0 ± 0.3	3.9 ± 0.1	4.1 ± 0.3	4.2 ± 0.1
Calcium (mg/dL)	7.9-12.0	10.4 ± 0.5	10.2 ± 0.4	10.2 ± 0.3	10.1 ± 0.4	10.1 ± 0.5
Inorganic phosphorus (mg/dL)	2.5-6.8	4.0 ± 0.5	4.4 ± 0.8	4.1 ± 0.6	3.9 ± 0.6	3.8 ± 0.3
Glucose (mg/dL)	77-125	93.8 ± 3.9	103.0 ± 8.0	90.4 ± 15.2	90.8 ± 16.6	86.4 ± 3.3

*Data are expressed as Mean ± SD of 5 dogs. *P < 0.05 vs. Control with one-way analysis of variance for repeated measures and a posteriori testing with Dunnett's multiple comparison test. WBC, White blood cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; A/G, albumin/globulin.*

RESULTS

All dogs showed no clinical signs for 30 days. No photosensitivity occurred. Serum creatinine was significantly different between control and 1 month after AO administration, but this variation was within the normal limit. Other results showed no significant differences between control and later time points, and the results of all serum chemistry were within normal limits (Table 1).

Time course changes in serum AO concentrations after IV administration of AO (0.1 mg/kg) are shown Fig 1. The serum AO

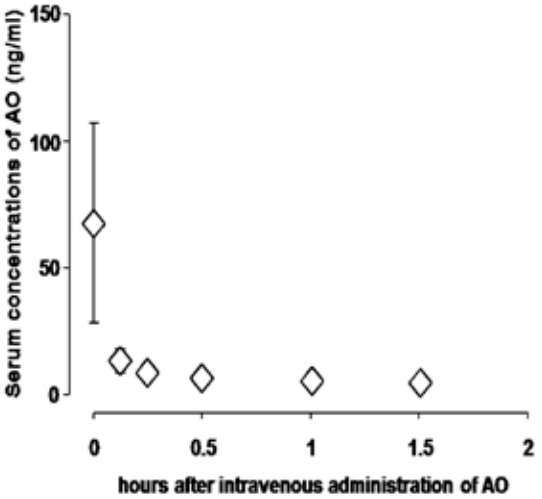

Figure. 1 *Time course changes in serum concentrations of acridine orange (AO) after intravenous administration of AO (0.1 mg/kg). Data are expressed as mean ± SD of 5 dogs. The serum AO level decreased rapidly and was below the detection limit (5 ng/mL) 2 hr after injection.*

level decreased rapidly, and it was below the detection limit 2 hr after injection.

DISCUSSION

In this study, IV administration of AO at a dose of 0.1 mg/kg was safe on a short-term basis, since there were no clinical signs and CBC and serum chemistry values were within normal limits. The serum AO concentration reached a peak immediately after administration, and it was below the detection limit 2 hr after injection.

Quinacrine hydrochloride, which is an acridine derivative, has been administered orally as an antiprotozoal drug, and its toxicities have been reported to date. In small animals, a yellow skin and urine color, gastrointestinal disturbances, abnormal behaviors, pruritus, and fever have been noted.[12] Quinacrine crosses the placenta and has been implicated in causing deformity in a human infant. [12] At high doses, it caused increased fetal death rates in rats. [12] Therefore, it was thought that AO, which is an acridine derivative, should not be administered to pregnant animals. IV administration of AO did not show any side effects such as gastrointestinal disturbances, abnormal behaviors, or pruritus. However, since other toxicity remains unclear, this approach should be limited to dogs with a short lifespan.

In general, PDT makes the skin and eyes sensitive to light for 6 weeks or longer after treatment.[13] Cutaneous photosensitization appears to be an uncommon problem in dogs and cats treated with PDT, which may reflect the limited use of porfimer sodium (Photofrin® Pinnacle Biologics) in these species, differences in photosensitizer distribution compared to humans, or differences in the skin and adnexa between species.[14] Photosensitization with systemic AO has not been reported.[6, 10, 11] In the present study, there was no evidence of photosensitivity. Thus, 0.1 mg/kg appears to be safe in dogs.

It was thought that the toxicity of this drug was mild. The International Agency for Research on Cancer (IARC) of the World Health Organization reported that this agent was considered to be not classifiable as to its carcinogenic (class 3).[15] Some authors reported usage of AO solution at surgical sites in humans,[3, 8] and no toxicity was reported. With systemic administration, this drug was safe at an oral dose of 500 mg in humans, and the only side effect was mild gastrointestinal symptoms (nausea 3 cases and vomiting 1 case out of 35 patients).[16] It has been reported that the LD_{50} of IV AO was 27.3 mg/kg in mice,[11] and that 1 to 10 mg/kg IV was safe in mice.[9, 10, 17] Satonaka et al. (2006) reported that AO at 0.1 mg/kg IV provided the best visual contrast on digital images.[11] Thus, in this study, AO 0.1 mg/kg was administered IV, and no clinical signs were detected. The serum AO concentration decreased rapidly after the initial peak following injection, and it was below the limit of detection 2 hr after the injection. Serum

creatinine was increased 1 month after AO administration, but it was within the normal limit (0.76 ± 0.09 mg/dL). Thus, this was not thought to be clinically significant.

In this study, IV administration of AO 0.1 mg/kg was safe on a short-term basis. Systemic administration of this drug should be limited to dogs with malignant tumors and a short lifespan, because the long-term effects of systemic AO are not known.

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