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Research Article

Study on Pharmic Effects of Anti-Inflammatory and Ease Pain of Arsenous Acid Injections

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Abstract

Objective: To explore anti-inflammatory and ease pain effects of Arsenous Acid (AA) injection.

Methods: Measurement of mouse ear swelling caused by dimethylbenzene of rat posterior peri metatarsal swelling in Freund's complete adjuvant arthritis and of relative weight of rat spleen, thymus, adrenal gland, iliac lymph nodes to the whole body, to assess the anti-inflammatory effects of AA injection; observation of the sedative effects of AA injection on acetic acid-caused mouse writhing.

Results: AA injection markedly inhibited acute inflammatory ear swelling following dimethylbenzene stimulation on mouse ear; therapeutic use of AA injection (4mg/kg) significantly decreased the swelling of adjuvant arthritis foot (right) and left (control) foots delayed allergic swelling on rat on the 5th and 8th treatment day; AA injection showed evident protective effects in adjuvant arthritis in scoring method, and markedly alleviated mouses writhing after acetic acid stimulation.

Conclusion: Arsenous Acid (AA) injection has anti-inflammatory and ease pain effects.

Keywords: Arsenous Acid Injection Anti-Inflammatory Ease Pain, Posterior Feet, Acute Promyelocytic Leukemia, Hydrocortisone, Piroxicam, Adrenal Gland, Iliac Lymph Nodes, Thymus, Acetic Acid.

Introduction

Since 1995, reports [1-5] on clinical and mechanism research of Arsenic Trioxide (As_2O_3) treatment of Acute Promyelocytic Leukemia (APL) has injected fresh vitality into the use of As_2O_3, a poisonous drug from traditional Chinese medicinal materials [6]. Over the recent years, researches on As_2O_3 or other arsenicals treatment of tumors became a hot spot, and suggested that arsenicals check tumor cell metabolism through interfering with sulfhydry1 enzymes, regulate tumor-associated genes and relevant processes, induce tumor cell differentiation or apoptosis and death, all of which constitute a composite anti-tumor mechanism [7]. As_2O_3 treatment makes long-term survivals possible for most of APL patients, with a high rate of complete links and only mild poisonous and side effects [7,8]. Arsenicals showed therapeutic effects not only on APL, but also on liver cancer and other solid carcinomas [9,10] probably playing a role of broad spectrum of anti-tumor treatment. Also, not long ago, a rheumatic arthritis patient, Wang XX, made routine dose intravenous drip of AA injection by himself and on his own initiative,3 days later felt an obvious relief of arthralgia and evident improvement of left elbow function. This case led us to look back on the previous literature records about arsenical treatment of rheumatism, and to make a

recent exploration of the anti-inflammatory and sedative effects of arsenicals.

Materials and Methods

Materials

a. Animals. Wistar rats of body weight 160-200g and Kunming mice of body weight 18-22g, were provided by animal experiment center of Shenyang University of Pharmacy. Animal quality certificate No.033 of Liaoning experimental animal management.

b. Granular forage made by Shenyang experimental animal forage factory.

c. Arsenous acid injection made by Harbin Yida pharmaceutical company, lot number 9901101

Other experimental drugs

a. Hydrocortisone made by Shenyang 1st pharmaceutical factory, No.002752 (1996) of Liaoning medicinal administration, lot number 990603.

b. BCG vaccine made by Changchun biological products institute of the Health Ministry, No, Changchun [1] 10 of health products (82). Lot number 20000506-1.

c. Piroxicam made by Liaodong pharmaceutical company of Jilin chemical company. Lot number 991001.

Experimental methods [11]

Anti-inflammatory experiment

Effect of AA injection on mouse ear swelling caused by dimethylbenzene: Mice of body weight 18-22g were divided randomly into five groups with 10 (sex ratio 1:1) in each group.

a. Solvent group animals were each injected into the abdominal cavity the same volume of normal saline as the AA injection group.

b. Arsenous Acid (AA)injection group animals were divided into 3 subgroups of doses: 1mg/kg, 2mg/kg, and 4mg/dg.

c. Hydrocortisone group animals were injected hydrocortisone, 4mg/kg, into the abdominal cavity.

Animal in all the groups were each given solution volume of 20ml/kg daily for 5 successive days. On day 5, at 1/2 hour after intra-abdominal infusion of AA injection, dimethy1 benzene in a dose of 50ul/animal was evenly smeared on both sides of mouse right ear with the left ear as control. After two hours, the mice were sacrificed by luxation method, a 7mm diameter round slice was taken, using a perforator, from the same sites of both cut ears, the slice weight was compared with the left slice as 100%, the swelling rate of the right ear and the inhibitory effectiveness of drug were recorded.

Therapeutic effects of Arsenous Acid (AA) injection on adjuvant arthritis of rat: Rats (sex ratio 1:1) of body weight 160-200g were used. The volumes of both posterior feet were measured using foot volume instrument. Then each animal was given subcutaneous injection of 0.1ml of Freund's complete adjuvant (containing 0.5mg of inactivated Mycobacterium tuberculosis) in the right posterior peri-metatarsal area. After 15 days, the whole-body weight, the volume of each posterior foot and arthritis scoring were recorded. The rats were divided into 6 groups with 10 animals in each group (based on left or control foot swelling and the whole-body weight) to perform the following experiments.

a. The solvent group animals were given intra-abdominal injection of the same volume of normal saline as in other groups.

b. AA injection in a dose of 0.5, 1.0, 2.0 or 4.0mg/kg was injected in four AA injection subgroups.

c. Intra-gastric perfusion of piroxicam (0.4mg/kg) in piroxicam group.

The intra-abdominal injection was given in a solution volume of 20 ml/kg daily for 7 successive days. The whole-body weight, posterior foot volume, arthritis scoring, foot swelling rate were recorded at days 2,5, and 8 after the beginning of drug experiment. At the day 9, the rats were sacrificed, the relative weights of the spleen, liver, thymus, adrenal gland, and iliac lymph nodes to the whole body were taken (mg/100g body weight).

Sedation. Effect of AA injection on the mouse writhing caused by acetic acid: Mice of body weight 18-22g were divided randomly into 6 groups with 10 animals (sex ratio 1:1) in each group.

a. Solvent group animals were each given intra-abdominal injection of normal saline as animals in other groups.

b. AA injection in a dose of 0.5, 1.0, 2.0 or 4.0mg/kg was injected in the four AA injecting subgroups.

c. Intra-gastric perfusion of piroxicam in a dose of 3mg/kg. The intra-abdominal drug injection was given in a solution volume of 20ml/kg. At 1/2 hour after abdominal injection, and at 1 hour after intra-gastric perfusion, 0.2ml (per mouse) of 0.6% acetic acid was injected intra-abdominally to record how many times each mouse writhed within 5 to 20 minutes after acetic acid injection.

Results

Anti-inflammatory effect

Three doses of AA injection showed evident inhibition on mouse ear swelling caused by dimethylbenzene, but without significant dose-effect relationship. The inhibition of AA injection on the mouse ear swelling was weaker than that of 4.0mg/kg of hydrocortisone (Table 1). In comparison with the solvent, AA injection in dose of 4.0mg/kg evidently decreased the swelling rate at days 5 and 8 after AA injection treatment, treatment, and in dose of 2.0 mg/kg showed only an insignificant decrease tendency of swelling; the efficacy of piroxicam at days 5 and 8 after using piroxicam was similar to that of 4.0 mg/kg of AA injection (p>0.05, student's t -test of difference (Table 2). On inhibition to delayed allergic swelling of the left foot (control foot not injected by Freund's adjuvant), 4.0 mg/kg of AA injection showed insignificant inhibition on day 2, but evident inhibition on days 5 and 8; and showed similar efficacy to that of piroxicam on days 5 and 8 (Table 3).

Table 1: Effect of AA injection on mouse ear swelling caused by dimethylbenzene.

Group	Dose (mg/kg/day)	No. of Animals	Swelling Rate (%) (X±SD)	Inhibition Rate (%)
Solvent	-----	10	117.02±28.54	
AA	1.0	10	81.50±34.98*	30.35
Injection	2.0	10	79.22±36.88**	32.3
	4.0	10	79.19±22.36**	32.33
Hydro-Cortisone	4.0	10	70.25±30.68**	39.07

Note: A*=p<0.05, **=p<0.01, B*=p<0.05**=p<0.05

Student's t-test, A in comparison with the solvent group. B. in comparison with the Hydrocortisone.

Table 2: Effect of AA injector on foot swelling of adjuvant arthritis foot.

Group	Dose (mg/kg/day)	No. of Animals	Swelling Rate (%) (x±sd)			
			1	2	5	8 (day)
Solvent		10	97.13±51.84	81.97±46.61	78.52±37.61	87.34±51.20
AA Injection	0.5	10	113.18±57.88	89.50±49.12	94.38±33.36	59.73±35.77
	1.0	10	88.34±40.59	74.85±33.70	74.53±29.64	65.61±31.00
	2.0	10	91.52±45.69	64.51±36.92	51.34±24.67	55.86±34.55
	4.0	10	71.03±42.86	53.36±30.46	42.96±28.24	42.50±20.75*
Piroxicam	0.4	10	72.59±25.98	41.64±21.11	47.36±24.38	43.47±32.54

Note: A*=p<0.05, Student's t-test, in comparison with the solvent. Swelling rate (%) (X±SD)

Table 3: Effect of AA injection on delayed allergic swelling of the left foot (control foot not injected by Freund's adjuvant).

Group	Dose (mg/kg/day)	No. of Animals	Swelling Rate (%) (X±SD)			
			1	2	5	8 (day)
Solvent		10	37.74±13.51	40.67±15.34	34.31±12.65	43.59±18.36
AA Injection	0.5	10	34.93±12.49	36.03±13.17	35.54±18.10	36.96±16.98
	1.0	10	30.20±14.30	17.72±10.13	24.70±11.53	22.44±10.62
	2.0	10	36.23±17.57	23.48±12.12	17.71±10.01	16.41±9.89
	4,0	10	24.76±11.82	17.67±9.24	8.30±4.43*	7.62±3.54
Piroxicam	0.4	10	19.87±10.04	18.04±9.97	8.36±5.12**	9.23±4.32

Note: A*= p<0.05, **=p<0.01 in comparison with the solvent group.

B*=P>0.05*=P>0.05, in comparison with Piroxicam group (Student's t- test).

Reviewing from the scoring of rats 'ear erythema, anterior feet swelling, posterior left (control) foot swelling and other relevant lesions, and in comparison, with the solvent, AA injection showed significant protective effects in adjuvant arthritis on the 2nd, 5th and 8th treatment day when used in dose of 2.0 or 4.0mg/kg; AA injection in dose of 1.0mg/kg and piroxicam also showed significant protective effects on the 5th and 8th treatment days. There was no significant difference between the AA injection treatment

and piroxicam (Table 4). There was on significant decrease in forage intake during the whole experimental period. No significant difference was found between AA injection groups, piroxicam group, and the solvent group in terms of rat body weight, relative weight of the spleen, thymus, and adrenal gland in comparison with the solvent group, AA injection groups had somewhat but not significant increase in the weight of iliac lymph nodes (P＞0.05, Student`s t-test) (Tables 5 & 6).

Table 4: Effect of AA injection on the scoring of adjuvant arthritis in rat.

Group	Dose (mg/kg/day)	No. of Animals	Swelling Rate (%) (X±SD)			
			1	2	5	8 (day)
Solvent	-----	10	4.70±1.34	5.30±1.83	5.31±1.79	4.8±1.03
AA Injection	0.5	10	4.5±2.07	3.80±1.77	5.20±2.94	4.60±2.53
	1.0	10	5.00±2.05	3.70±2.11	3.30±1.49*	2.50±1.37**
	2.0	10	3.70±1.42	3.60±0.97	2.50±1.35**	2.10±1.21**
	4.0	10	3.90±1.37	3.30±1.34	3.00±1.25**	2.50±1.18**
Piroxicam	0.4	10	4.00±1.63	4.60±1.51	2.90±1.45**	2.00±1.41**

Note: *=P＜0.05, **P＜0.01, Student`s t-test, in comparison with the solvent group.

Table 5: Effect of AA injection on the body weight of rats with adjuvant arthritis.

Group	Dose (mg/kg/day)	No. of Animals	Body Weight (%) (X±SD)			
			1	2	5	8 (day)
Solvent	-----	10	259.0±49.3	256.0±39.6	254.0±53.6	268.0±68.0
AA Injection	0.5	10	265.0±40.1	262.0±30.8	254.0±42.0	263.0±40.0
	1.0	10	255.0±40.7	251.0±40.1	249.0±38.43	254.0±43.9
	2.0	10	257.5±35.4	245.0±43.3	223.2±117.9	255.0±47.0
	4.0	10	268.0±37.9	254.0±38.4	268.0±50.7	268.0±38.5
Piroxicam	0.4	10	259.0±44.3	277.0±39.5	259.0±51.3	268.0±52.5

Table 6: Effect of AA injection on the relative organ weight in rats with adjuvant arthritis (mg/100g of body weight).

Group	Dose (mg/kg/day)	No. of Animals	Spleen	Thymus	Adrenal Gland	Iliac Lymph Nodes
Solvent	-----	10	0.475±0.14	161.95±69.08	22.98±10.09	16.11±10.94
AA Injection	0.5	10	0.411±0.10	129.20±47.84	22.56±9.86	20.59±11.96
	1.0	10	0.445±0.20	106.51±37.02	20.62±7.41	26.88±13.68
	2.0	10	0.451±0.16	133.70±39.09	23.69±7.59	32.03±15.98
	4.0	10	0.378±0.19	114.50±27.82	23.89±9.91	21.48±12.83
Piroxicam	0.4	10	0.387±0.07	146.41±46.57	23.66±6.69	14.60±7.61

Note: p>0.05, Student's t – test, in comparison between the solvent group and each of treatment groups.

Sedation

Three dose groups of AA injection showed evident inhibition on mouse writhing caused by acetic acid, but with no significant dose-effect relationship. There was no significant difference between piroxicam treatment and the AA injection treatment (p>0.05, Student's t -test) (Table 7).

Table 7: Effect of AA injection on mouse writhing caused by acetic acid.

Group	Dose(mg/kg/day)	No. of Animals	No. of Writhing(X±SD)	Inhibition Rate (%)
Solvent	-----	10	37.20±9.52	
AA Injection	1.0	10	19.60±12.78**	47.31
	2.0	10	19.10±7.89**	48.66
	4.0	10	18.10±12.31**	51.34
Piroxicam	0.4	10	16.30±7.10*	56.18

Note: *= p < 0.05, ** = p<0.01, Student's t-text, in comparison with the solvent group.

Discussion

Freund's complete adjuvant arthritis in rat has pathological features like those of human rheumatoid arthritis. The process of this adjuvant arthritis can be divided into two stages the primary foot swelling following the peri-metatarsal adjuvant injection is non-allergic inflammatory reaction, begins several hours after the injection, reaches the peak degree after 4 to 6 hours and then gradually subsides; about 14 days later, the secondary swelling of the injected foot , the swelling of the control foot (not injected with the adjuvant) and the secondary changes in the tail and forefoot joints are allergic inflammatory in nature, relating with antigen-antibody complex and other delayed allergic factors [12,13]. Starting from the 15th day after Freund's complete adjuvant injection, therapeutic use of AA injection in a dose of 4.0 mg/kg showed evident inhibitory effectiveness on swelling of both sides as shown by the records of the 5th and 8th treatment days; and this anti-allergic effect was like that of piroxicam (Tables 2 & 3). The arthritis scoring (Table 4) also demonstrated that the protective effects of AA injection on the lesions of adjuvant arthritis are notable and like those of piroxicam, and that the AA injection has inhibit0ory and therapeutic effects on the immuno-inflammatory or delayed allergic reactions following Freund's adjuvant injection. These findings can probably provide several experimental bases for exploring AA injection use in treatment of immune arthritis. The observation that during the experimental period there developed no apparent changes in rat's forage intake, body weight, and relative weight of various immune organs suggests AA injection to be relatively safe in terms of poisonous and side effects (Tables 5 & 6). The results of the experiment also show that AA injection has obvious inhibitory effects on rat ear's acute inflammatory swelling caused by dimethylbenzene and no mouse writhing following intra-abdominal injection of acetic acid (Tables 1 & 7). In conclusion, Arsenous Acid (AA) injection has comparatively remarkable anti-inflammatory and ease pain therapeutic effectiveness.

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