Journal of Ethnopharmacology, 34 (1991) 215–219
Elsevier Scientific Publishers Ireland Ltd.

Radiation-protective and platelet aggregation inhibitory effects of five traditional Chinese drugs and acetylsalicylic acid following high-dose γ-irradiation

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(Accepted May 16, 1991)

High doses of ^{60}Co radiation (4.0–8.0 Gy) in mice, rats and rabbits caused increases in rate of platelet aggregation during the first 5 days after irradiation. The inhibitory effects of the extracts of five Chinese drug plants and acetylsalicylic acid on rate of platelet aggregation were observed in both in vitro and in vivo tests, averaging 23–53% in vitro and 46–69% in vivo. Antiradiation tests on mice vs. 7.5–8.0 Gy of γ-radiation, using the plant extracts and acetylsalicylic acid as protective agents, increased survival rates by 8–50% for the plant extracts and 35% for acetylsalicylic acid.

Key words: radiation protection; platelet aggregation inhibition; Chinese drugs.

Introduction

High doses of ionizing radiation bring about considerable disturbance to the microcirculatory system, causing microthrombosis and hemorrhage (Tian, 1984). These radiation effects are believed to be connected to platelet function, therefore increased platelet aggregation would be expected following irradiation. The tendency of platelet aggregation to increase during the first few days following γ-irradiation of animals has been investigated in the present study. At the same time, the effects of several platelet aggregation inhibitors on the extent of platelet aggregation following γ-irradiation have been documented. Platelet aggregation inhibitors employed include acetylsalicylic acid and five Chinese natural products known to have this property. The same substances were also used in radiation protection experiments to determine if inhibition of platelet aggregation confers some degree of radiation protection to animals.

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Materials and Methods

Preparation of plant extracts

The following quantities of ground Chinese drug plants were used: flowers of *Carthamus tinctorius* L. (Compositae), 100 g; stems of *Sargentodoxae cuneata* (Oliv.) Rehd. and Wils. (Sargentodoxaceae), 250 g; roots of *Paeonia lactiflora* (Pall.) (Ranunculaceae), 250 g; roots of *Salvia miltiorrizha* (Bge.) (Labiatae), 250 g; and rhizomes of *Ligusticum chuanxiong* (Hort.) (Umbelliferae), 250 g. Each dried material was mixed well and soaked with 100 ml of distilled water for 30 min (for *C. tinctorius* flowers, 600 ml was used). Each preparation was decocted twice (first for 1 h, and then for 30 min) and filtered after each decoction. The filtrates for each were combined

0378-8741/$03.50

and concentrated in a rotary evaporator until the fluid became viscous. Twice the volume of 95% ethanol was added, and the preparation was stored in the refrigerator for several days to allow precipitation. The precipitates were filtered, washed with 95% ethanol, and the combined filtrate and washings combined and again concentrated to a viscous mass. This was washed with 300–500 ml of 95% ethanol, and the washings were stored in the refrigerator for 2–3 days. The resulting mixture was filtered, and the filtrate was concentrated in the rotary evaporator to give a residue with no odor of ethanol. Yields of the individual plant extracts (% w/w) were less than 1%. Each residue was then diluted with distilled water to give a concentration of 100 mg/ml (equivalent to weight of starting material) for *C. tinctorius*, and 1 g/ml for the other plant extracts. All of these solutions were heated on a steam bath for 30 min, stored in the refrigerator for 2 days, and filtered through activated charcoal. This procedure was repeated twice. The pH of the filtrate then equaled 6.8. The filtrate was diluted with 3% glucose solution to give the required dilutions used in the tests, and the resulting solution was filtered through a No. 3 sintered glass funnel to give a transparent solution. This was boiled at 100°C for at least 30 min for sterilization.

Platelet aggregation following γ-irradiation

Six-week-old male mice of Kun Ming and ICR strains, 10-week-old male rats, and 2.0–2.5-kg New Zealand rabbits were used. Groups of 10 mice, 9–10 rats, and 11–13 rabbits were whole-body irradiated with ^{60}Co γ-rays. The mice were irradiated with a dose of 6.0 Gy at 2.03–2.08 Gy/min, the rats with a dose of 8.0 Gy at 2.0 Gy/min, and the rabbits with a dose of 4.0 Gy at 0.99 Gy/min. Platelet aggregation rates were determined at five different times, following irradiation, from +4 h to +7 days.

The response of platelet aggregation rate to irradiation was measured in a PAM-2 autoequilibrated aggregometer. Nine parts of blood were anticoagulated with one part of 3.8% sodium citrate solution and centrifuged at 110–240 × *g* for 3 min to prepare platelet-rich plasma (PRP), and at 850–1450 × *g* for 7 min to prepare platelet-poor plasma (PPP). To 0.2 ml of PRP (number of platelets = 2.0–2.5 × 10^5/mm^3) was added 10 μl of ADP (Sigma Chemical Co.) to give a final concentration of 1 μM for mice, 5 μM for rats, and 6 μM for rabbits. The rate level after radiation was compared with that prior to radiation in rabbits, and a comparison of rates in irradiated and non-irradiated mice and rats was made. Blood was obtained from the hearts of the rabbits, and from the common carotids of the mice and rats.

Inhibition of platelet aggregation

Observation of inhibition of platelet aggregation was made both in vitro and in vivo. The in vitro observations were done by adding the extracts to cups containing PRP (number of platelets = 2.0–3.0 × 10^5/mm^3) from rabbit heart. These experiments were repeated six times for an average result. The in vivo experiments were carried out by obtaining blood from a tube inserted in the common carotid of mice 30 min after the last i.p. injection of aqueous solutions of 10–100 mg of drug extract. The extracts were administered i.p. daily on days 1, 2, 3 and 4 after γ-ray irradiation. Control mice received injections of an equal volume of water. A daily dose of 0.5 mg/mouse for acetylsalicylic acid was given orally on days 1 and 2 (blood was withdrawn 2 h after the last dose), and 100 mg/mouse for the plant extracts, given i.p. on days 1, 2, 3 and 4 after irradiation.

Radiation protection by inhibitors of platelet aggregation

Radiation-protective effects were based on 30-day survival rates of mice irradiated with 7.5 and 8.0 Gy of ^{60}Co γ-rays. Protective compounds included the five plant extracts as well as acetylsalicylic acid. The acetylsalicylic acid was administered orally to a group of 20 mice in a dose of 0.3 mg on days 1 and 2 following irradiation. The extracts of the plant drugs were given to groups of 20 or 40 mice by i.p. injection on days 1–4 following irradiation.

Results

The data using mice, rats and rabbits show that high-dose γ-rays (4–8 Gy) can cause an increase in

TABLE 1

CHANGES OF PLATELET AGGREGATION RATES OF MOUSE, RAT AND RABBIT AFTER γ-RADIATION

Parentheses indicate the number of observations used to calculate the tabular mean ± S.E.M.

Animal	Radiation dose (Gy)	Control aggregation time (s)	Aggregation time (s)				
			+1/6	+1	+3	+5	+7 days
Mouse	6.0	26.2 ± 2.3 (10)	42.0 ± 3.3** (10)	26.9 ± 2.6 (10)	33.8 ± 3.5** (10)	33.0 ± 3.7** (10)	22.5 ± 4.3 (10)
Rat	8.0	22.8 ± 2.0 (18)	33.5 ± 1.0** (10)	42.8 ± 4.4** (10)	15.6 ± 3.0 (9)	31.2 ± 2.6* (9)	29.9 ± 4.9 (9)
Rabbit	4.0	31.4 ± 2.0 (13)	ND	40.3 ± 2.2* (11)	36.4 ± 3.0 (12)	46.8 ± 5. 7* (12)	29.4 ± 2.9 (13)

Statistically significant from respective control aggregation time: $*P < 0.05$, $**P < 0.01$.

TABLE 2

EFFECT ON PLATELET AGGREGATION OF CHINESE PLANT EXTRACTS IN RABBIT BLOOD IN VITRO (MEAN ± S.E.M.)[a]

Extract (plant part)	Concentration of extract (mg/ml)	*N*	Rate of aggregation (s)[b]		Inhibition (%)	*P*
			no drug	drug		
C. tinctorius (flowers)	2.27	6	28.8 ± 3.9	21.7 ± 3.3	24.8	> 0.05
S. cuneata (stems)	4.55	6	27.7 ± 2.6	21.2 ± 1.3	23.5	< 0.05
P. lactiflora (roots)	6.82	6	32.5 ± 3.3	19.2 ± 1.4	41.0	< 0.01
S. miltiorriza (roots)	13.64	6	34.2 ± 2.6	17.5 ± 1.9	49.0	< 0.01
L. chuanxiong (rhizomes)	6.82	6	29.4 ± 3.6	13.7 ± 2.4	53.4	< 0.01

[a]Inducing agent: ADP (6μM). [b]Number of platelets: 2.0–3.0 × 10^5/mm^3.

TABLE 3

EFFECT ON PLATELET AGGREGATION BY ACETYLSALICYLIC ACID AND CHINESE PLANT EXTRACTS IN ICR MOUSE BLOOD IN VIVO (MEAN ± S.E.M.)[a]

Treatment	*N*	Dose (mg/mouse)	Rate of aggregation (s)[b]	Inhibition (%)
Saline	6	(0.2 ml)	40.0 ± 6.0	—
C. tinctorius	6	30	14.0 ± 4.7**	65.0
S. cuneata	6	50	13.6 ± 6.4**	66.0
P. lactiflora	6	100	12.5 ± 7.7**	68.8
S. miltiorrizha	6	100	21.4 ± 7.8*	46.5
L. chuanxiong	6	100	14.6 ± 8.0**	63.5
Acetylsalicylic acid[c]	6	0.5	13.8 ± 7.8**	65.5

[a]Inducing agent: ADP (1 μM).
[b]Number of platelets: 2.0–3.0 × 10^5/mm^3.
[c]Measurement of platelet aggregation rate for acetylsalicylic acid was done 2 h after last oral administration, while measurement for the extracts was 30 min after last i.p. dose.
Significant from saline control: $*P < 0.05$, $**P < 0.01$.

rate of platelet aggregation during the first 5 days after irradiation (Table 1). A marked rise in the response rate in mice and rats 4 h after radiation was followed by a fall on the first day in mice, but was maintained at a high level in rats until the third day. The rate in mice rose significantly on the third and fifth days. The reduction in rate in rats on the third day was followed by an increase on the fifth and a small decrease on the seventh. In rabbits there was an increase in rate of aggregation on the first and fifth days with a drop on the third day. A similar pattern of increase, decrease, increase and decrease during the 7-day period was found for all three species.

An inhibiting effect of the plant extracts on platelet aggregation in rabbit blood in vitro was demonstrated for all five plant species (Table 2). The reductions in rate observed ran from 23–53%, measured 6 min after addition of the drugs. In the in vivo experiments in mice, acetylsalicylic acid inhibited the rate of aggregation by 65%, while the plant extracts caused reductions of 63–69%, with the exception of the *S. miltiorrhiza* root extract, which caused a reduction of 46.5%. Data are shown in Table 3.

Antiradiation effects of acetylsalicylic acid and the plant extracts were demonstrated by observing the 30-day survival rates of mice irradiated with 7.5–8. 0 Gy. The survival rate of mice protected with acetylsalicylic acid against 7.5 Gy was 35% more than that of its respective control group. *C. tinctorius* extract increased survival rate by an insignificant 7.5%, but the other plant drugs raised survival rates by 20–50%. Life spans of the deceased animals were increased in each case, and the protective coefficient (average life span of treated/controls) ranged from 1.09 to 1.55 for those treated with the plant extracts, compared to 1.24 for those treated with acetylsalicylic acid (Table 4).

Discussion

Platelet hyperaggregation is one of the common manifestations of certain hemopathologic and cardiovascular diseases. Inhibitors of platelet aggregation have been found effective for these disease states (Seuter, 1984). Table 1 shows increases in platelet aggregation in the mouse, rat and rabbit following irradiation with 4.0–8.0 Gy doses of γ-radiation. For mice and rats, this increase is evident within 4 h after radiation, and is maintained for 5 days in all three animal species. Wustrow et al. (1983) have reported that an increased aggregation rate in mice was already significant within 2 h after a combination of radiation and other injuries. Stosick (1975) has also demonstrated that platelet aggregation rate increased very soon after radiation therapy in human patients using radium. An increase in platelet aggregation rate in two leukemia patients

TABLE 4

PROTECTIVE EFFECTS OF ACETYLSALICYLIC ACID AND CHINESE PLANT EXTRACTS ON MICE IRRADIATED WITH 7.5–8.0 Gy γ-RADIATION

Treatment	Dose (mg/mouse)	Survivors (quantal)		Change in survival rate (%)	Average life-span of dead mice (days)		Protective coefficient
		Controls	Treated		Controls	Treated	
Acetylsalicylic acid	0.3	5/20	12/20	+35.0*	8.9	11.0	1.24
C. tinctorius	10.0	4/40	7/40	+7.5	11.1	12.0	1.09
S. cuneata	100.0	4/40	13/40	+22.5*	11.1	15.4	1.40
P. lactiflora	12.5	0/10	4/10	+40.0*	11.5	15.6	1.35
P. lactiflora	25.0	0/10	2/10	+20.0	11.5	19.5	1.70
S. miltiorrizha	30.0	0/20	4/20	+20.0	11.5	14.2	1.23
L. chuanxiong	100.0	4/40	12/20	+50.0*	11.1	13.5	1.22

Significant prolongation of life: $*P < 0.01$.

following whole body radiation therapy with γ-rays has also been observed (Wang et al., 1985). The rate of aggregation in one patient exposed to 6 Gy increased from 27% pre-irradiation to 78% on day 3 and to 49% on day 5. In the other case, the response of platelet aggregation from 20% pre-irradiation to 51% was observed 6 h following 1.4 Gy, and to 44% 8 h after 4.5 Gy.

The mechanism by which radiation increases the rate of platelet aggregation is not known. Baluda et al. (1981) have shown that the effect of blood vessel walls in inhibiting the increased aggregation was reduced immediately after γ-irradiation of mice. They also found that irradiation of rats and guinea pigs reduced prostacyclin-like activity in the abdominal aorta wall. Both prostacyclin and thromboxanes, which induce platelet aggregation, are thus involved in the process of hyperaggregation through lesions in the vascular endothelium (Wang, 1986).

Increased platelet activity is probably mainly responsible for the microthrombosis blocking the microcirculation following high doses of ionizing radiation. In addition, it can lead to the serious bleeding observed at vascular wall lesions because of exhaustion of platelets and coagulation factors through hyperaggregation. Inhibition of platelet aggregation, therefore, is of value in the repair of damage resulting from sizeable doses of radiation.

It is well known that acetylsalicylic acid is an inhibitor of platelet function. A small dose in mice (0.5 mg/mouse) gave a 65% reduction in rate of platelet aggregation 2 h after the last dose (Table 3). It also increased the survival rate of irradiated mice (7.5 Gy) by 35% (Table 4). The five plant extracts also gave reductions in the rate of platelet aggregation in mice of over 63%, with one exception (46.5% for *S. miltiorrizha*), 30 min after the last injection. The increase in survival rate in mice, after irradiation (7.5 Gy), by the plant extracts ranged from 7.5% to 50%. It may be concluded that inhibitors of platelet aggregation may be useful for the treatment of acute radiation injury during the first few days following irradiation.

Acknowledgment

The authors thank Dr. X.D. Li for authenticating the plant materials.

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