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Effects of a Novel Pyridylsulphonyl Thiazole Derivative, FR115092, on Autoimmune and Mitomycin C-induced Thrombycytopenia in Mice

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Abstract

Dapsone (4,4′-diaminodiphenyl sulphone), an antileprotic and antimalarial drug, has been reported to be of therapeutic benefit in idiopathic thrombocytopenic purpura in the clinic. However, adverse reactions such as haemolytic anaemia have often been observed. In this study, we found that dapsone increased the number of platelets and decreased the number of red blood cells in male (NZW×BXSB)F_1 (W/BF_1) mice, an animal model of idiopathic thrombocytopenic purpura. In studies to prepare derivatives of dapsone with weaker side effects than the parent compound, FR115092 (2-[5-(2-pyridylsulphonyl)thiazolyl]amine) was discovered. The effect of FR115092 on the number of blood cells was studied and compared with dapsone in mice.

FR115092 increased the number of platelets without reducing the number of red blood cells in W/BF_1 mice. This drug significantly suppressed the increase in circulating autoantibodies against platelets and increased the number of megakaryocytes. Furthermore, FR115092 inhibited the reduction of the number of platelets in mitomycin C-induced thrombocytopenic mice, as a consequence of its enhancement of growth and maturation of megakaryocytes.

These findings suggest that FR115092 may be effective against various thrombocytopenias, without inducing haemolytic anaemia.

Idiopathic thrombocytopenic purpura (ITP) is considered to be an autoimmune disorder characterized by thrombocytopenia which is caused by anti-platelet autoantibodies. Autoantibodies binding to platelet surface antigens, glycoprotein (GP)Ib and GPIIb/IIIa (Woods et al 1984; Neiman et al 1987; Fujisawa et al 1993), are removed by the function of the reticulo-endothelial system in the spleen and liver (McMillan 1981; Karpatkin 1985), and consequently the number of platelets is reduced in peripheral blood. Although various therapeutic approaches, for example, vinca alkaloids, danazol, immunosuppressive agents and high-dose intravenous immunoglobulins, have been used in ITP patients, a more effective regimen without adverse effects remains to be defined (Bussel 1990; Jubelirer 1993).

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Dapsone (4,4′-diaminodiphenyl sulphone) is an antileprotic and antimalarial drug. In addition, this drug has been found to be beneficial in certain cutaneous inflammatory disorders in man (Ruzicka & Goerz 1981; Tsutsui et al 1996). Recently, the beneficial effect of dapsone in patients with refractory ITP and other chronic thrombocytopenia has been reported (Moss & Hamilton 1988; Robert & Jim 1989; Durand et al 1991a, b, 1993; Godeau et al 1993; Linares et al 1994; Hernandez et al 1995; Lesprit et al 1995; Wynn et al 1995). Treatment with dapsone for a few weeks significantly increased the number of platelets in the peripheral blood of the patients. This response was similar to, or better than, those obtained with other therapeutic approaches, although dapson often induced haemolysis, which limited the period of treatment and the dose level. Thus, a derivative of dapsone that does not induce haemolytic anaemia would be useful in the treatment of ITP. In an effort to find a compound with weaker haemolytic side

effects than dapsone, we synthesized a number of derivatives of dapsone and selected FR115092 (2-[5-(2-pyridylsulphonyl)thiazolyl]amine) as a candidate for development (Ogino et al 1998).

The male (NZW×BXSB)F_1 (W/BF_1) mouse is a model for ITP and lupus nephritis with myocardial infarction (Oyaizu et al 1988; Mizutani et al 1990; Ikehara et al 1995). The number of platelets in male W/BF_1 mice is significantly reduced with age, with an increase in the number of platelet-associated autoantibodies (PAAs) (Ikehara et al 1995).

In this study, the effect of FR115092 on the reduction of the number of platelets was compared with that of dapsone in W/BF_1 mice. Also, the effects of FR115092 on platelet production were studied in antitumour drug-induced thrombocytopenic mice.

Materials and Methods

Mice

Male mice of the W/BF_1 strain (hybrids of NZW females and BXSB males) and female mice of ddY strains were purchased from Japan SLC (Shizuoka, Japan).

Drugs

Dapsone and mitomycin C were purchased from Tokyo Kasei Kogyo Co. Ltd (Tokyo, Japan) and Kyowa Hakko Kogyo Co. Ltd (Tokyo, Japan), respectively. FR115092 was prepared in our laboratories (Ogino et al 1998). The chemical structures of dapsone and FR115092 are shown in Figure 1. Dapsone and FR115092 were suspended in 0·5% methylcellulose, and given orally in a volume of 10 mL kg^{-1} body weight. Mitomycin C was dissolved in and diluted with saline, and given intravenously in a volume of 10 mL kg^{-1} body weight.

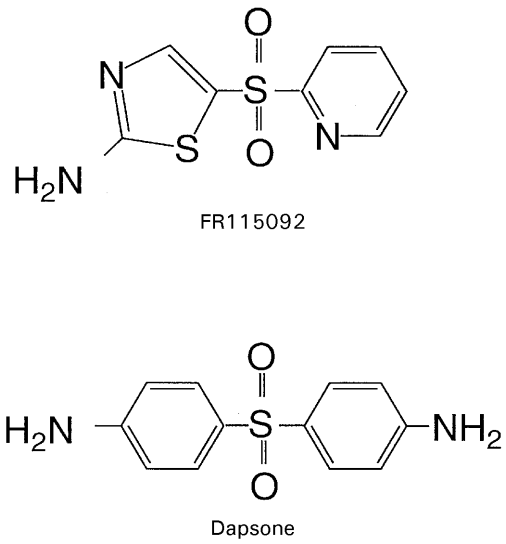

Figure 1. Structures of FR115092 and dapsone.

Peripheral blood cell counts

Peripheral blood anticoagulated with EDTA-4Na was obtained from intracardiac puncture under ether anaesthesia. The number of platelets and red blood cells was counted using a Sysmex E-4000 (Toa Medical Electronics Co., Kobe, Japan).

Measurement of PAAs

A two-colour stained immunofluorescence test was used for PAAs according to the methods of Mizutani et al (1990). Briefly, blood (100 μL) was suspended in 1·0 mL 1% paraformaldehyde solution (pH 7·4) for 2 h. After washing three times with 5 mM EDTA-2Na in phosphate-buffered saline (PBS), blood cells were incubated with 0·5 μL fluorescein isothiocyanate (FITC) conjugated goat anti-mouse Ig antibody (PharMingen, San Diego, CA), 1·25 μL *R*-phycoerythrin conjugated hamster anti-mouse CD61 antibody (PharMingen) and 48·25 μL PBS for 15 min at room temperature. After washing twice with PBS, samples were resuspended in PBS and analysed on a FACScan (Becton Dickinson, Sunnyvale, CA). Intensity of fluorescence was expressed as the mean channel of the histogram for the quantification of PAAs.

Number of bone marrow cells

Mice were killed by cervical dislocation. Both ends of the femur were cut out aseptically. Bone marrow cells were then flushed with a syringe using a 26-gauge needle with 1·0 mL 10% foetal bovine serum (FBS) (Intergen, Purchase, NY)-CATCH medium. The CATCH medium was composed of 129 mM NaCl, 8·6 mM Na_2HPO_4, 1·6 mM KH_2PO_4, 13·6 mM Na citrate, 11·1 mM glucose, 1 mM adenosine, 2 mM theophylline and 2·3 μM prostaglandin E_1 (Yonemura et al 1992). The pH was adjusted to 7·0. The bone marrow cells were collected by centrifugation, resuspended in lysis buffer (0·14 M NH_4Cl, 17 mM Tris, pH 7·2) and incubated for 10 min at 37°C. After incubation, the bone marrow cells were counted by Sysmex E-4000.

Number of megakaryocytes

After washing the bone marrow cells with 10% FBS-CATCH, the cells were incubated with FITC

conjugated hamster anti-mouse-CD61 antibody (FITC-CD61) (PharMingen) for 20 min at room temperature. After washing twice with PBS, the cells were incubated with 50 μg mL^{-1} propiodium iodide (Sigma Chemical Co., St Louis, MO) in 0·1% Na citrate for 2 h at 4°C. Megakaryocytes were selected by staining with FITC-CD61 and the number counted in 10^6 bone marrow cells by FACScan.

Number of colony-forming units-megakaryocyte (CFU-meg) colony

A fibrin clot culture system was used to count the number of CFU-meg colonies (Kuriya et al 1987). Briefly, 0·2 mL of bone marrow cell suspension (1×10^6 cells) was added to a solution containing 0·4 mL FBS, 0·4 mL pockweed mitogen-stimulated mouse spleen cell-conditioned medium, 0·4 mL double-strength Iscove's modified Dulbecco's medium (IMDM) (Sigma), 0·4 mL bovine fibrinogen solution (2·5 mg mL^{-1} in 0·1 M phosphate buffer, pH 7·1, Sigma) and 0·2 mL bovine thrombin solution (2 units mL^{-1} in IMDM; Sigma). The cells (2×6 mL IMDM was added around the clot. After seven days incubation, in a fully humidified atmosphere of 5% CO_2 in air at 37°C, the fibrin clots were fixed with 5% glutaraldehyde, stained in-situ for acetylcholinesterase, and counterstained with Harris' haematoxylin using the method described by Kuriya et al (1987). Aggregates of eight or more acetylcholinesterase-positive nucleated cells were scored as megakaryocyte colonies using a Nikon OPTIPHOT microscope (Tokyo, Japan).

DNA ploidy in megakaryocytes

Measurement of DNA ploidy of megakaryocytes was performed by FACScan analysis as reported (Jackson et al 1984). Briefly, bone marrow cells stained by FITC-CD61 and propiodium iodide were used in the distribution analysis of the megakaryocyte ploidy. On FACScan analysis, megakaryocytes were used as FITC-CD61 positive cells. Ploidy distribution of megakaryocytes was determined by setting markers at the lowest value between the peaks of each DNA content (2N, 4N...). The percentage of the number of megakaryocytes with each DNA content was calculated by FACScan.

Statistical analysis

Differences were evaluated by using the one-way analysis of variance and Dunnett tests.

Results

Effects of FR115092 and dapsone on platelet and red blood cell counts in W/BF$_1$ mice

It has been reported that oral treatment with dapsone increases the number of platelets in refractory ITP patients. Therefore, the haematopoietic effects of FR115092 and dapsone were examined in W/BF$_1$ mice. The number of platelets in W/BF$_1$ mice spontaneously decreases in an age-dependent relationship. In 5-week-old W/BF$_1$ mice, the number of platelets was normal ($98·3\pm4·2\times10^4$ μL^{-1}). In 15-week-old W/BF$_1$ mice the number of platelets had declined to half the normal value ($52·2\pm18·2\times10^4$ μL^{-1}) and had reached the lowest value in 20-week-old mice ($23·1\pm3·0\times10^4$ μL^{-1}).

FR115092 or dapsone was given orally to 16-week-old mice twice a day for six weeks. The results are shown in Table 1. FR115092 increased the number of platelets dose-dependently, and at a dose of 100 mg kg^{-1} platelet counts significantly

Table 1. Effects of FR115092 and dapsone on platelet counts, red blood cell counts and platelet-associated autoantibodies (PAAs) in W/BF$_1$ mice.

Treatment	Number of mice	Platelets ($\times10^4$ cells μL^{-1})	Red blood cells ($\times10^4$ cells μL^{-1})	PAAs (% of control)
Control	23/30	23·8 ± 3·5	769 ± 28	100·0 ± 13·0
FR115092 10 mg kg^{-1}	14/20	29·0 ± 4·4	817 ± 13	96·4 ± 13·5
FR115092 32 mg kg^{-1}	29/29	35·2 ± 3·9	756 ± 14	69·8 ± 5·6
FR115092 100 mg kg^{-1}	24/29	43·7 ± 4·7*	785 ± 21	56·7 ± 7·2*
Dapsone 10 mg kg^{-1}	15/20	26·1 ± 2·8	731 ± 32	105·6 ± 12·0
Dapsone 32 mg kg^{-1}	26/27	44·7 ± 4·4**	703 ± 9	110·7 ± 10·4
Dapsone 100 mg kg^{-1}	8/29	51·1 ± 9·8*	634 ± 42*	58·5 ± 13·8

Drugs were given orally to mice twice a day from the age of 16 weeks to 21 weeks. Control mice received 0·5% methylcellulose. On the day after the final treatment, platelets and red blood cells were counted by Sysmex E-4000, and PAAs were analysed using FACScan. The number of mice is shown as number of surviving mice/number of mice used. Values are mean ± s.e. *$P < 0·05$, **$P < 0·01$ compared with the control group.

increased, although the number of red blood cells remained unchanged. Dapsone-treated mice showed a dose-dependent increase in the number of platelets, but also a dose-dependent decrease in the number of red blood cells. Dapsone at 100 mg kg^{-1} caused death in 72% of the mice.

PAAs after treatment with FR115092 or dapsone in W/BF$_1$ mice

FR115092 and dapsone increased the number of platelets in the peripheral blood of W/BF$_1$ mice. PAAs were measured as a possible indicator of the mechanism of action. The PAAs value was expressed as percent of mean channel of fluorescence intensity of the treated group to that of the control group on FACScan analysis. FR115092 or dapsone was given orally to 16-week-old mice twice a day for six weeks. The results are shown in Table 1. FR115092 significantly and dose-dependently decreased the level of PAAs, and the effect was in parallel with the increase in the number of platelets, suggesting that the level of PAAs in blood is, at least in part, important for induction of the effect of FR115092 on the number of platelets. However, 100 mg kg^{-1} dapsone decreased the level of PAAs, which increased the number of platelets. Dapsone at 32 mg kg^{-1} increased the number of platelets, but it did not decrease the level of PAAs in W/BF$_1$ mice.

Effects of long-term treatment with FR115092 and dapsone on W/BF$_1$ mice

Seven-week-old W/BF$_1$ mice (platelet count: $118{\cdot}5 \pm 7{\cdot}1 \times 10^4$ μL^{-1}) were administered FR115092 or dapsone orally once a day for 18 weeks. The results are shown in Table 2. FR115092 and dapsone at doses of 32 and 100 mg kg^{-1} increased the number of platelets. Dapsone also decreased the number of red blood cells, but FR115092 did not. FR115092 and dapsone at 32 mg kg^{-1} increased the number of platelets to a level more than 2-fold of that in mice treated with 0·5% methylcellulose (control), without reducing the level of PAAs. When the number of platelets decreases to a level less than 30% of control, it may induce pathological disease like haemorrhage. Both drugs increased the number of platelets to levels of more than 30% of the control group, suggesting recovery from haemorrhage. This phenomenon prompted us to examine the effects of the two drugs on the number of megakaryocytes in bone marrow, which is the precursor cell for platelets. FR115092 at 32 and 100 mg kg^{-1} was found to increase the number of megakaryocytes. Although 32 mg kg^{-1} dapsone slightly increased the number of megakaryocytes, at 100 mg kg^{-1} the number decreased.

Effects of FR115092 on the number of platelets and red blood cells in mitomycin C-treated mice

Since FR115092 was found to increase the number of platelets in peripheral blood, and the number of megakaryocytes in the bone marrow of W/BF$_1$ mice, it is likely that the drug would be effective on the decrease of platelet counts in mice with myelosuppression. The effect of FR115092 was thus examined in mice with myelosuppression induced by the cancer chemotherapeutic drug, mitomycin C. The results are shown in Figure 2. When mitomycin C (3·2 mg kg^{-1}, i.v.) was administered to ddY mice on days 0, 2 and 4, the number of platelets was markedly decreased and reached the lowest point (12% of the control) on day 15 (Figure 2A). When FR115092 was given orally to mitomycin C-treated mice twice a day on days 0, 1, 2, 3 and 4, the 32 and 100 mg kg^{-1} doses reduced the decrease in the number of platelets induced by mitomycin C and counts quickly recovered to normal values. The number of platelets on day 22 in the FR115092 (32 and 100 mg kg^{-1})-treated

Table 2. Effects of long term treatment with FR115092 and dapsone on platelet counts, red blood cell counts, platelet-associated autoantibodies (PAAs) and megakaryocyte counts in W/BF$_1$ mice.

Treatment	Number of mice	Platelets ($\times 10^4$ cells μL^{-1})	Red blood cells ($\times 10^4$ cells μL^{-1})	PAAs (% of control)	Megakaryocytes ($\times 10^2$ cells/femur)
Control	9/14	22·8 ± 3·5	728 ± 32	100·0 ± 16·3	90·2 ± 20·5
FR115092 32 mg kg^{-1}	9/14	44·7 ± 11·0	729 ± 31	100·2 ± 30·6	118·3 ± 26·8
FR115092 100 mg kg^{-1}	13/14	53·7 ± 8·4*	689 ± 39	69·9 ± 10·9	138·8 ± 22·8
Dapsone 32 mg kg^{-1}	11/14	52·7 ± 8·0	653 ± 57	87·9 ± 37·9	109·8 ± 33·0
Dapsone 100 mg kg^{-1}	11/14	66·2 ± 9·0**	605 ± 32	39·1 ± 6·1	63·7 ± 18·3

Drugs were given orally to W/BF$_1$ mice once a day from the age of 7 weeks to 24 weeks. Control mice received 0·5% methylcellulose. On the day after the final treatment, platelets and red blood cells were counted by Sysmex E-4000, and PAAs and the number of megakaryocytes were analysed using FACScan. The number of mice is shown as number of surviving mice/number of mice used. Values are mean ± s.e. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared with the control group.

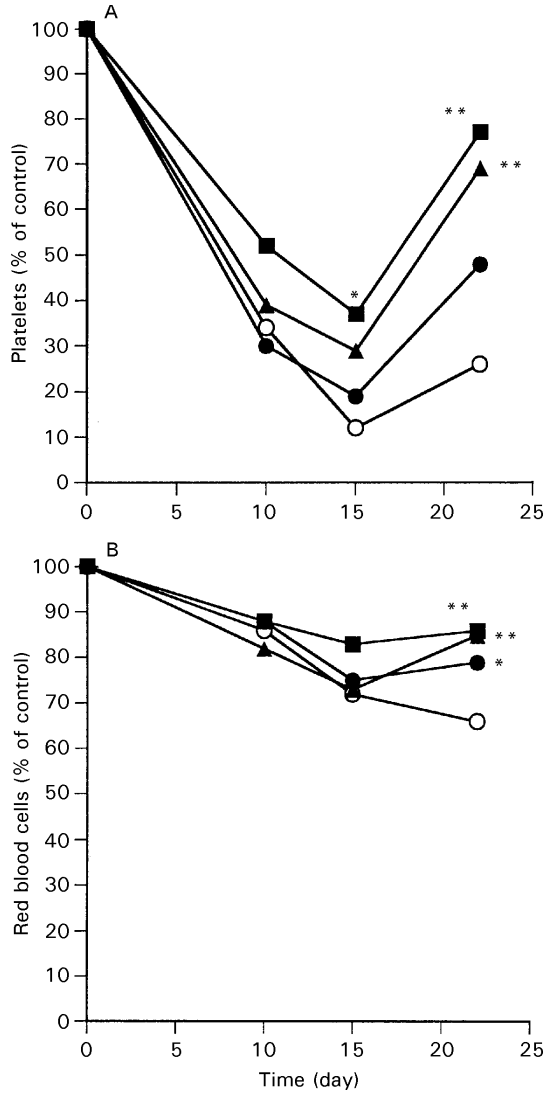

Figure 2. Effects of FR115092 on platelet (A) and red blood cell counts (B) in mitomycin C-induced thrombocytopenic mice. Mitomycin C (3·2 mg kg^{-1}, i.v.) was given to mice on days 0, 2 and 4. FR115092 (○ 0, ● 10, ▲ 32, and ■ 100 mg kg^{-1}) was given orally to mice twice a day on days 0, 1, 2, 3 and 4. Platelets and red blood cells were counted on days 10, 15 and 22 by Sysmex E-4000. Values are mean ± s.e., n = 10. *$P < 0.05$, **$P < 0.001$ compared with the mitomycin C group.

mice was about 69-77% of the number in untreated mice (Figure 2A).

The number of red blood cells gradually decreased in the mitomycin C-treated mice. FR115092 did not further reduce the number of red blood cells in mitomycin C-treated mice, by day 22 the numbers had increased (Figure 2B).

Thrombopoietic effects of FR115092 in mitomycin C-treated mice

Mitomycin C (3·2 mg kg^{-1}, i.v.) was given to ddY mice on days 0, 2 and 4, and FR115092 (100 mg kg^{-1}, p.o.) was given twice a day on days 0, 1, 2, 3 and 4. On day 18, bone marrow cells were collected from the femur of the mice, and the numbers of bone marrow cells and megakaryocytes were counted using FACScan. The results are shown in Table 3. The number of bone marrow cells in the mitomycin C-treated mice was $88 \pm 16 \times 10^5$/femur, similar to the control mice. The number of bone marrow cells in the FR115092 and mitomycin C-treated mice was $109 \pm 11 \times 10^5$/femur, 124% of that in mitomycin C-treated mice. Next, the number of CFU-meg in bone marrow cells was counted. In mitomycin C-treated mice, the number of CFU-meg was reduced to 21% that of the control mice. FR115092 induced good recovery of CFU-meg to 80% of the control in the mitomycin C-treated mice. Furthermore, mitomycin C significantly reduced the number of megakaryocytes in bone marrow to 41% of control, and a combination of FR115092 and mitomycin C increased the number to 78% of control.

The cellular DNA content of megakaryocytes was measured, using FACScan, to investigate the effects of FR115092 on the maturation of megakaryocytes in bone marrow. In the control group, megakaryocytes with 16N content were the major part (51% of megakaryocytes) (Figure 3A). When mice were treated with mitomycin C, 16N and 32N cells were significantly reduced (7% and 12%,

Table 3. Effects of FR115092 on number of bone marrow cells, number of megakaryocytes, and CFU-meg of femurs in mitomycin C-treated mice.

Treatment	Bone marrow cells ($\times 10^5$ cells/femur)	CFU-meg (colonies/femur)	Megakaryocytes ($\times 10^2$ cells/femur)
Control	82 ± 9	105 ± 4**	85 ± 11**
Mitomycin C 3·2 mg kg^{-1}	88 ± 16	22 ± 3	35 ± 11
FR115092 100 mg kg^{-1} + mitomycin C 3·2 mg kg^{-1}	109 ± 11	84 ± 2**	66 ± 6

Mitomycin C (3·2 mg kg^{-1}, i.v.) was given on days 0, 2 and 4. FR115092 (100 mg kg^{-1}, p.o.) was given to mice twice a day on days 0, 1, 2, 3 and 4. Control mice received saline and 0·5% methylcellulose. On day 18 bone marrow cells, CFU-meg and megakaryocytes were counted as described in Materials and Methods. Mice were used in groups of five. Values are mean ± s.e. **$P < 0.01$ compared with the mitomycin C group.

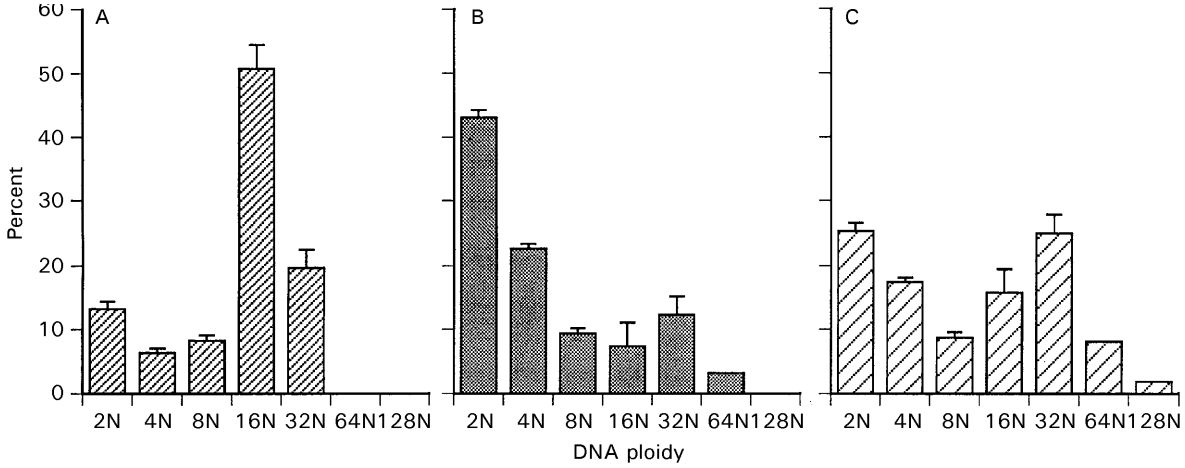

Figure 3. Effects of FR115092 on megakaryocyte DNA ploidy distribution in mitomycin C-induced thrombocytopenic mice. Mitomycin C (3·2 mg kg^{-1}, i.v.) was given to mice on days 0, 2 and 4. FR115092 (100 mg kg^{-1}, p.o.) was given to mice twice a day on days 0, 1, 2, 3 and 4. On day 18 bone marrow cells were collected from the femurs of mice and megakaryocyte DNA ploidy was analysed by FACScan. A. Control, B. mitomycin C 3·2 mg kg^{-1}, C. FR115092 100 mg kg^{-1} + mitomycin C 3·2 mg kg^{-1}. Bars represent mean ± s.e., n = 5.

respectively) and 2N cells increased to 43% of megakaryocytes and formed a major peak (Figure 3B). In mice treated with FR115092 and mitomycin C, 16N and 32N cells increased to 16% and 25%, respectively, and 2N cells decreased to 25%. Cells with 128N ploidy were also observed in this group (Figure 3C). The results suggest that FR115092 blocks the de-maturation of megakaryocytes induced by mitomycin C.

Discussion

ITP is reported to be a disease of the reduction of platelets in peripheral blood resulting from an increase in anti-platelet autoantibodies (Karpatkin 1980). Steroids and splenectomy are often used for the therapy of ITP. Although a suitable animal model for ITP has not been found yet, male W/BF$_1$ mice have been reported to develop thrombocytopenia spontaneously with age and show a good reverse correlation between the number of platelets and PAAs levels (Oyaizu et al 1988; Mizutani et al 1990). In male W/BF$_1$ mice, prednisolone has been reported to increase the number of platelets by decreasing PAAs levels and suppression of the function of the reticulo-endothelial system, especially in the spleen and liver, which led to its clinical effectiveness in thrombocytopenia (Mizutani et al 1992a). Splenectomy increased platelet counts and reduced PAAs in W/BF$_1$ mice (Mizutani et al 1992b). As prednisolone and splenectomy both have elicited a good response in W/BF$_1$ mice, these mice may be a superior ITP model that reflects human ITP disease. Dapsone increases the number of platelets in the peripheral blood of ITP patients, but often induces haemolytic anaemia (Durand et al 1991a, b; Godeau et al 1993; Linares et al 1994; Wynn et al 1995). Therefore dapsone must be used with special care when used for ITP therapy in the clinic.

In this study, an increase of platelet counts was observed with FR115092- and dapsone-treated W/BF$_1$ mice, even when the treatment was started in 16-week-old W/BF$_1$ mice whose platelet counts were half that of normal mice. FR115092 increased the platelet counts to a level twice (approx.) that of untreated mice. These results suggest that FR115092 could be effective in ITP patients, as efficacious as dapsone. In the clinic, prednisolone is usually proposed as the first-line of treatment, but its side effects are inevitable. Splenectomy is effective but traumatic. Dapsone-induced haemolytic anaemia is also a problem. Thus, FR115092, which is effective against W/BF$_1$ mice without reducing the number of red blood cells, and which does not induce haemolytic anaemia, may be a suitable drug for refractory ITP. In clinical trials, the effects of dapsone in ITP patients were observed at doses between 50 and 100 mg. In our study, dapsone at doses of 32 and 100 mg kg^{-1} increased the number of platelets in the animal model. It is evident that to induce the effects of dapsone in animals, much larger doses are needed than for patients. This might be because the syndromes of ITP are rapidly induced in W/BF$_1$ mice compared with patients.

The precise mechanism by which dapsone increases the number of platelets remains to be clarified. Similarly, the mode of the action of FR115092 on the number of platelets is unknown.

It is thought that ITP is an autoimmune disease, whereby autoantibodies against platelets are produced and autoantibody-coated platelets are destroyed by a function of the reticulo-endothelial system (McMillan 1981; Karpatkin 1985). First, we measured PAAs as autoantibodies against platelets in W/BF_1 mice, and found that FR115092 and dapsone dose-dependently decreased PAAs levels. Thus, one of the mechanisms to increase platelet number in FR115092- or dapsone-treated W/BF_1 mice was thought to be reduction of PAAs.

Reduction of PAAs, however, can not completely explain the effects of FR115092. Long-term treatment with FR115092 at a dose of $32\,mg\,kg^{-1}$, which did not decrease PAAs, resulted in an increase in the number of platelets. Therefore, the effect of FR115092 on production of platelets was examined. Stem cells differentiate into committed megakaryocyte progenitors and the megakaryocytes are matured by polyploidization and cytoplasmic maturation (megakaryocytopoiesis). Finally, the matured megakaryocytes release a number of platelets. The production is regulated by the interplay of various cytokines and bone marrow stromal cells (Metcalf et al 1991; Laterveer et al 1993; Waring et al 1993; Yonemura et al 1993; Bartley et al 1994; Kaushansky et al 1994; Lok et al 1994; Wendling et al 1994; Zeigler et al 1994). We found that FR115092 led to an increase in the number of megakaryocytes in W/BF_1 mice, but did not increase the number of bone marrow cells (data not shown). These results indicate that FR115092 might stimulate the release of specific growth factors or maturation factors against megakaryocytes or activate them. Although interleukin-6 (Laterveer et al 1993), leukaemia inhibiting factor (Metcalf et al 1991; Waring et al 1993), interleukin-11 (Yonemura et al 1993) and thrombopoietin (Bartley et al 1994; Kaushansky et al 1994; Lok et al 1994; Wendling et al 1994; Zeigler et al 1994) have been reported to be growth or maturation factors for platelets, the possibility of megakaryocyte activating factors regulated by FR115092 has not been confirmed. These findings suggest that FR115092 induced an increase in the number of platelets in W/BF_1 mice, due to two different mechanisms: reduction of circulating PAAs levels and enhancement of megakaryocyte growth. Although dapsone did not increase the numbers of bone marrow cells and megakaryocytes, its effect on the ITP mice model was very similar to that of FR115092. Dapsone decreased the number of red blood cells in W/BF_1 mice, and the number of platelets increased with reduction in the number of red blood cells. The destruction of red blood cells mainly occurs by a function of the reticulo-endothelial system in liver and spleen (Baxter et al 1985). It is likely that excessive red blood cell destruction may induce maximum activation of the reticulo-endothelial system function and the reticulo-endothelial system function for other blood cells may be inhibited, which reduces the removal of autoantibodies binding to platelets and increases the number of platelets. Thus, dapsone-induced reduction of PAAs and suppression of the reticulo-endothelial system function may play a role in the induction of the effects of dapsone on thrombocytopenia in W/BF_1 mice.

Since cancer chemotherapeutic drugs such as mitomycin C mainly act on cells which grow rapidly, it shows cytotoxicity against bone marrow cells as well as tumour cells, leading to myelosuppression. The fact that FR115092 increased the number of megakaryocytes in W/BF_1 mice led us to examine the effects of FR115092 on cancer chemotherapeutic agent-induced thrombocytopenia. In this study, we found that FR115092 inhibited the reduction of the number of platelets in mitomycin C-induced thrombocytopenic mice. The characteristics of the actions of FR115092 were an increase of platelet counts and rapid recovery from the lowest values. These effects were not due to the release of stored platelets in the spleen, because FR115092 also increased the number of platelets in mitomycin C-induced thrombocytopenic mice that had been splenectomized (data not shown). Promotion of growth of bone marrow cells and megakaryocytes, and maturation of megakaryocytes were considered as mechanisms of action for FR115092 in mitomycin C-induced thrombocytopenic mice. Thrombopoietin has also been reported to promote recovery in mitomycin C-induced thrombocytopenic mice (Akahori et al 1996). The rapid recovery of platelet counts by FR115092 may be due to augmentation of growth factors for megakaryocytes. However, the precise mechanism is not clear. FR115092 inhibited mitomycin C-induced reduction of red blood cell counts. Promotion of growth of bone marrow cells may also be considered as one of the mechanisms of FR115092. Although FR115092 inhibited reduction of platelets in the mitomycin C-treated mice, it did not diminish the antitumour effects of mitomycin C against murine tumours implanted in mice (data not shown). These results suggest that the effect of FR115092 on thrombocytopenia is not based on suppression of the antitumour action of mitomycin C.

FR115092 had no effect on the number of platelets in normal mice (data not shown), although the drug increased the number of platelets in W/BF_1 mice and mitomycin C-treated mice. The

results suggest that FR115092 induces thrombopoietic effects in mice under the conditions of thrombocytopenia. FR115092 increased the number of platelets in W/BF_1 mice, not only by suppressing auto-platelet antibody production, but also by inducing the growth of megakaryocytes. Additionally, the drug was effective on mitomycin C-induced thrombocytopenia by induction of growth and maturation of megakaryocytes. FR115092 may be an effective drug for ITP and cancer chemotherapy-induced thrombocytopenia.

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