Anti-Cancer Drugs, 2, pp. 139–143

Dipyridamole combination chemotherapy can be used safely in treating gastric cancer patients

Yoshihisa Sakaguchi,[CA] Yoshihiko Maehara, Yasunori Emi, Tetsuya Kusumoto, Shunji Kohnoe and Keizo Sugimachi

Y Sakaguchi, Y Emi, T Kusumoto, S Kohnoe and K Sugimachi are at the Cancer Center of Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan. Tel: 010-81-92-641-1151, ext. 2781; fax: 010-81-92-632-3001. Y Maehara and K Sugimachi are at the Department of Surgery II, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

The feasibility of a combined chemotherapy using dipyridamole (DP) with adriamycin (ADM) and 5-fluorouracil (5-FU) was investigated. First, the chemosensitivity of gastric cancer tissues was determined by the succinate dehydrogenase inhibition test, which showed sensitivity to ADM and 5-FU is increased by DP. Next, a clinical trial of combined therapy of DP, ADM and 5-FU, as a post-operative adjuvant chemotherapy for gastric cancer patients, was performed. DP (50 mg) was given as a 1-h iv infusion, and ADM (20 mg) was given as a single iv injection. This treatment was started on post-operative day 10, and was repeated every 2 weeks. Simultaneously with these treatments, DP (300 mg) and 5-FU (150 mg) were administered post-operatively daily. A total of 63 courses of therapy in nine patients were performed. The adverse effects related to the DP infusion were flushing, headache, nausea and upper abdominal discomfort, all of a low grade. DP did not appear to alter the toxicity of ADM and 5-FU, and no severe adverse effect was noted for this combination therapy. The pharmacokinetics of DP were also investigated in five patients. The mean plasma concentration of DP increased 4.41 μg/ml and remained above 0.25 μg/ml for over 6 h. This combination chemotherapy appears to be safe and may be useful clinically in treating cancer.

Key words: Dipyridamole, adriamycin, 5-fluorouracil, SDI test.

Introduction

Dipyridamole (DP) is a vasodilator, an antiplatelet agent and a potent inhibitor of membrane nucleoside transport and cyclic AMP phosphodiesterase.[1] DP enhances the cytotoxicity of various anti-cancer drugs: acivicin,[2] *N*-phosphonacetyl-L-aspartate (NPLA),[3] methotrexate,[4] vincristine,[5] vinblastine[6] and etoposide.[6] Phase I clinical trials of DP have been performed in combination with NPLA,[7] acivicin[8] and methotrexate.[9] Since the clinical pharmacology of DP is well known and it has no serious toxicity in humans,[10] DP has received much attention as a biochemical modulator of anti-cancer drugs.

Grem and Fischer[11,12] showed that DP potentiates the cytotoxicity of 5-fluorouracil (5-FU) in a dose- and time-dependent manner. We reported previously that adriamycin (ADM) was synergistic in combination with DP both *in vitro* and *in vivo*, and that DP increased the intracellular accumulation of ADM in a dose- and time-dependent manner.[13]

On the bases of these preclinical results, we studied the chemosensitivity of gastric cancer tissues to ADM or 5-FU in combination with DP. As post-operative adjuvant chemotherapy for gastric cancer patients, we prescribed DP in combination with ADM and 5-FU, and investigated the pharmacokinetics of DP. The objective of this clinical trial was to determine the safety and usefulness of this combination of drugs.

Materials and methods

In vitro study

Chemosensitivity test. The chemosensitivity of gastric cancer tissues was determined using the succinate dehydrogenase inhibition (SDI) test.[14,15] The drugs used in this test were ADM (4 μg/ml), 5-FU (100 μg/ml) and DP (2.5 μg/ml). Gastric cancer tissue specimens were obtained from 10 patients

[A] Corresponding Author

who underwent gastrectomy in the Department of Surgery II, Kyushu University Hospital, Fukuoka, Japan. These specimens were first placed in McCoy's 5A solution, cut with scissors, passed through a No. 32 stainless steel mesh into McCoy's 5A solution containing antibiotics, and subsequently washed three times with this solution. The tissue fragments were then suspended in minimal essential medium with L-glutamine (292 mg/ml), 10% fetal calf serum and antibiotics, plated in each of 35 mm plastic dishes (3 dishes per test group and 4–6 in control), and then incubated while exposed to the drug at 37°C in a humidified 5% CO_2 atmosphere for 3 days. These fragments were then assayed for succinate dehydrogenase (SD) activity. Three - (4,5 - dimethyl - 2 - thiazolyl) - 2,5 - diphenyl - ^{2}H-tetrazolium bromide (MTT)[16] was used as the hydrogen acceptor for the SD activity. The formazan formed from MTT was extracted with acetone containing 0.5% trichloroacetic acid and its absorbance was measured at 565 nm. The chemosensitivity was estimated by the percentage of SD activity compared with that of control cells.

Clinical study

Patients. This clinical trial was conducted in nine Japanese patients with gastric cancer who had been surgically treated in the Department of Surgery II of the Kyushu University Hospital. They had neither symptomatic coronary artery disease nor any coagulation disorder. All patients gave informed consent.

Study design. Ten days after the operation, the patients were given their first course of combined chemotherapy. DP was administered in a dose of 50 mg/body in a 250-ml volume of normal saline as a 1-h iv infusion, and ADM was administered in a dose of 20 mg/body as a single iv bolus injection 30 min after initiating the DP infusion. This treatment course was repeated every 2 weeks. Simultaneously with this treatment, DP and 5-FU were administered post-operatively daily for as long as possible, as maintenance therapy. The patients received 300 mg/day of DP (100 mg every 8 h) and 150 mg/day of 5-FU (50 mg every 8 h).

Evaluation of adverse effects. During the DP infusion the patients were observed carefully and their blood pressure and pulse were monitored. Complete blood cell counts and white blood cell differential counts, liver and renal function tests and urinalysis were carried out every 2 weeks. The electrocardiogram was examined monthly.

Pharmacokinetics study. In five patients, blood samples were collected at 15, 30, 45, 60, 90, 120, 360 min and 24 h after initiating the DP infusion in the first course, at the time when oral administration of DP had not begun. The plasma concentration of DP was measured by high performance liquid chromatography as adapted from published methods.[17]

Results

Chemosensitivity of gastric cancer tissues

The chemosensitivities of gastric cancer tissues to ADM or 5-FU were compared to those to ADM plus DP or 5-FU plus DP using the SDI test. DP alone did not affect SD activity. By adding DP, SD activity decreased beyond that expected with a single use of either ADM or 5-FU (Figure 1). Thus, DP was found, *in vitro*, to increase the sensitivity of gastric cancer tissues to these anti-cancer drugs.

Patient characteristics

Nine patients were entered in this trial to receive combination chemotherapy with DP (Table 1). They included eight men and one woman, with a mean age of 61.9 (range 30–80) years. Three of them had undergone non-curative resection and the others had undergone curative resection. A total of 63 courses of therapy were performed. The greatest number of courses given was 17. The doses of DP administered by infusion ranged from 29.9 to 36.5 mg/m^2.

Adverse effects

DP infusion was associated with nausea in one case, flushing and headache in another case, and upper abdominal discomfort in a third case (Table 1). These symptoms occurred during the infusion of DP, subsided spontaneously after termination of its administration, and required no treatment. No significant changes in blood pressure or pulse, due to DP infusion, were noted. Myelosuppression was mild. Only one patient had leukopenia with a WBC nadir below 3000/m^3. Thrombocytopenia never

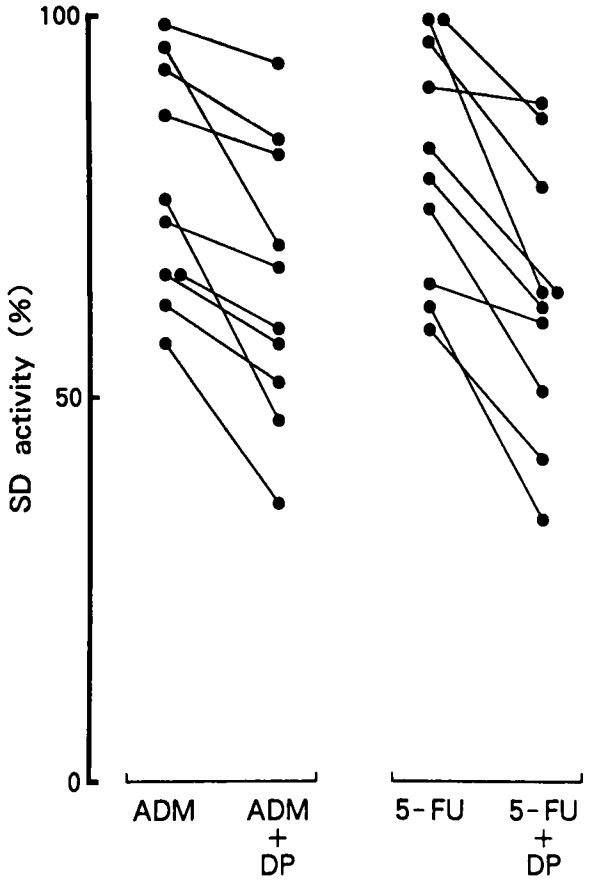

Figure 1. Chemosensitivity to ADM and 5-FU alone or in combination with DP. The chemosensitivity of gastric cancer tissues was investigated using the SDI test. The SD activity is expressed as a percentage of the control.

occurred throughout the study. Alopecia was seen in one case. There was little hepatic and renal toxicity due to the combination chemotherapy in this trial, and no abnormal change was seen on any electrocardiogram. The oral administration of DP and 5-FU was well tolerated. There was no severe adverse effect in this combination chemotherapy, which might have obligated us to discontinue the study.

Pharmacokinetics of DP

The plasma concentrations of DP were monitored in five patients during the first course in which DP was administered by infusion alone. As shown in Figure 2, the mean plasma DP concentration increased linearly until 30 min after initiating the infusion to reach 4.41 ± 1.01 μg/ml and then decreased gradually. A plasma DP level of at least 0.25 μg/ml was maintained for over 6 h.

Discussion

DP has been found to modulate the effects of various anti-cancer drugs.[1–6] It is also known from preclinical experiments *in vitro* and *in vivo* that DP enhances the cytotoxicity of both ADM and 5-FU.[11–13,18] The clinical efficacy of DP against cancer, however, is unknown. In this study, we demonstrated that DP increased the sensitivity of gastric cancer tissues to ADM and 5-FU when DP was given together with these drugs, as measured with the SDI test. These findings indicate that the prescription of DP with ADM or 5-FU may benefit patients with gastric cancer.

Since DP has been used safely as a vasodilator and an antiplatelet agent without serious toxicity,[10] it is likely to be safe as a biochemical modulator of

Table 1. Patient characteristics

Case	Age (years)	Sex	Operation	Course[a]	Dose of DP (mg/m²)[b]	Adverse effect[c]
1	77	Male	Curative	2	31.6	(−)
2	63	Male	Curative	4	32.4	(−)
3	77	Male	Curative	4	33.8	(−)
4	30	Male	Curative	5	32.3	(−)
5	80	Male	Non-curative	5	35.7	Upper abdominal discomfort
6	53	Female	Curative	7	36.5	Flushing, Headache
7	61	Male	Non-curative	8	31.2	Nausea
8	56	Male	Non-curative	11	29.9	(−)
9	59	Male	Curative	17	33.8	(−)

[a] Number of courses of DP infusion.
[b] Dose of DP administered by infusion iv.
[c] Adverse effects due to DP infusion.

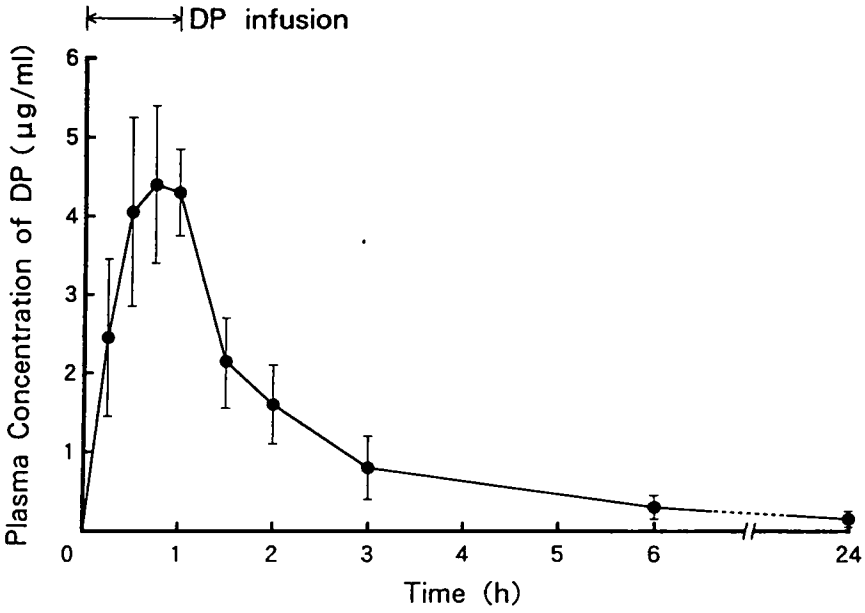

Figure 2. Plasma concentrations of DP. The pharmacokinetics of DP were determined during the first course of DP infusion in five patients. ●—Mean plasma concentration; bars—SD.

anti-cancer drugs for patients with cancer. In our clinical study, a 1-h infusion of 50 mg of DP was associated with the symptoms of flushing, headache, nausea and upper abdominal discomfort. These complaints were similar to those reported in clinical trials of combination chemotherapy with DP and anti-cancer drugs.[8,9,19] However, these adverse effects of DP infusion were not severe and required no treatment. Although higher doses of DP tend to increase the incidence of adverse effects (Table 1), we consider that a DP dose of up to 35 mg/m^2 can be administered safely as a 1-h infusion. Moreover, DP does not augment the systemic toxicity of either ADM or 5-FU. Remick *et al.*[19] also showed that DP did not augment the clinical toxicity of 5-FU in their phase I trial of combination therapy with 5-FU and DP.

DP enhances the cytotoxicity of ADM by increasing the intracellular accumulation of ADM.[13] Howell *et al.*[6] showed that DP reduces the efflux of ADM in a human ovarian carcinoma cell line. The mechanism by which DP enhanced the effects of 5-FU was thought to be that DP inhibits thymidine salvage and prevents the efflux of 5-fluorodeoxyuridine 5′-monophosphate.[12] Considering these mechanisms, it is necessary to maintain the plasma concentration of DP at effective levels for a long period of time. With a 1-h infusion of 50 mg of DP, the plasma DP concentration was kept above 0.25 μg/ml (0.5 μM), the concentration required to enhance the cytotoxicity of antitumor drugs *in vitro*,[11,13] for over 6 h.

Conclusion

We conclude that DP has potential as a biochemical modulator of anti-cancer drugs and is expected to be useful for clinical combination treatment. A 1-h infusion is considered to be an adequate method to administer DP. The combination of ADM, 5-FU and DP shows promise for more adequate results of cancer chemotherapy.

Acknowledgment

We thank M. Ohara for comments.

References

1. Harker L, Kadatz RA. Mechanism of action of dipyridamole. *Thromb Res* 1983; **4**: 39–46.
2. Fischer PH, Pamukcu R, Bittner G, Willson JKV. Enhancement of the sensitivity of human colon cancer cells to growth inhibition by acivicin achieved through inhibition of nucleic acid precursor salvage by dipyridamole. *Cancer Res* 1984; **44**: 3355–9.
3. Chan TCK, Howell SB. Mechanism of synergy between *N*-phosphonacetyl-L-aspartate and dipyridamole in a

human ovarian carcinoma cell line. *Cancer Res* 1985; **45**: 3598–604.
4. Cabral S, Leis S, Bover L, *et al.* Dipyridamole inhibits reversion by thymidine of methotrexate effect and increases drug uptake in Sarcoma 180 cells. *Proc Natl Acad Sci USA* 1984; **81**: 3200–3.
5. Hirose M, Takeda E, Nomiyama T, *et al.* Synergistic inhibitory effects of dipyridamole and vincristine on the growth of human leukaemia and lymphoma cell lines. *Br J Cancer* 1987; **56**: 413–7.
6. Howell SB, Hom D, Sanga R, *et al.* Comparison of the synergistic potentiation of etoposide, doxorubicin, and vinblastine cytotoxicity by dipyridamole. *Cancer Res* 1989; **49**: 3178–83.
7. Markman M, Chan TCK, Cleary S, Howell SB. Phase I trial of combination therapy of cancer with *N*-phosphonacetyl-L-aspartic acid and dipyridamole. *Cancer Chemother Pharmacol* 1987; **19**: 80–3.
8. Willson JKV, Fischer PH, Tutsch K, *et al.* Phase I clinical trial of a combination of dipyridamole and acivicin based upon inhibition of nucleoside salvage. *Cancer Res* 1988; **48**: 5585–90.
9. Willson JKV, Fischer PH, Remick SC, *et al.* Methotrexate and dipyridamole combination chemotherapy based upon inhibition of nucleoside salvage in humans. *Cancer Res* 1989; **49**: 1866–70.
10. The Persantine–Aspirin Reinfarction Study Research Group. Persantine and aspirin in coronary heart disease. *Circulation* 1980; **62**: 449–61.
11. Grem JL, Fischer PH. Augmentation of 5-fluorouracil cytotoxicity in human colon cancer cells by dipyridamole. *Cancer Res* 1985; **45**: 2967–72.
12. Grem JL, Fischer PH. Alteration of fluorouracil metabolism in human colon cancer cells by dipyridamole with a selective increase in fluorodeoxyuridine monophosphate levels. *Cancer Res* 1986; **48**: 6191–9.
13. Kusumoto H, Maehara Y, Anai H, *et al.* Potentiation of adriamycin cytotoxicity by dipyridamole against HeLa cells *in vitro* and Sarcoma 180 cells *in vivo*. *Cancer Res* 1988; **48**: 1208–12.
14. Anai H, Maehara Y, Kusumoto H, Sugimachi K. Comparison between succinate dehydrogenase inhibition test and subrenal capsule assay for chemosensitivity testing. *Oncology* 1987; **44**: 115–7.
15. Maehara Y, Anai H, Kusumoto H, Sugimachi K. Poorly differentiated human gastric carcinoma is more sensitive to antitumor drugs than is well differentiated carcinoma. *Eur J Surg Oncol* 1987; **13**: 203–6.
16. Cole SPC. Rapid chemosensitivity testing of human lung tumor cells using the MTT assay. *Cancer Chemother Pharmacol* 1986; **17**: 259–63.
17. Wolfram KM, Bjornsson TD. High performance liquid chromatographic analysis of dipyridamole in plasma and whole blood. *J Chromatog* 1980; **183**: 57–64.
18. Sakaguchi Y, Emi Y, Maehara Y, *et al.* Combined treatment of adriamycin and dipyridamole inhibits lung metastasis of B16 melanoma cells in mice. *Eur Surg Res* 1990; **22**: 213–8.
19. Remick SC, Grem JL, Fischer PH, *et al.* Phase I trial of 5-fluorouracil and dipyridamole administered by seventy-two-hour concurrent continuous infusion. *Cancer Res* 1990; **50**: 2667–72.

(Received 29 January 1991; accepted 15 February 1991)