Isobologram

The dose-response interactions between pemetrexed and paclitaxel for the MCF7, PA1 and WiDr cells were evaluated at the IC_{80} level by the isobologram method (Fig. 1) [32]. The IC_{80} was defined as the concentration of drug that produced 80% cell growth inhibition, i.e., an 80% reduction of absorbance. Since the A549 cells were resistant to pemetrexed and the IC_{80} level was not obtained, the interactions between pemetrexed and paclitaxel were evaluated at the IC_{50} level. We used the isobologram method of Steel and Peckham because this method can cope with any agents with unclear cytotoxic mechanisms and a variety of dose-response curves of anticancer agents [32]. The concept of the isobologram has been described in detail previously [11, 16].

Three isoeffect curves, mode I and mode II, were constructed, based upon the dose-response curves of pemetrexed and paclitaxel (Fig. 1). Mode I and mode II were generated by the assumption regarding overlap and non-overlap damage in combinations, respectively. Thus, when the data points of the drug combination fell within the area surrounded by mode I and/or mode II lines (i.e., within the envelope of additivity), the combination was described as additive. We used this envelope not only to evaluate the simultaneous exposure combinations of pemetrexed and paclitaxel, but also to evaluate the sequential exposure combinations, since the second agent under our experimental conditions could modulate the cytotoxicity of the first agent.

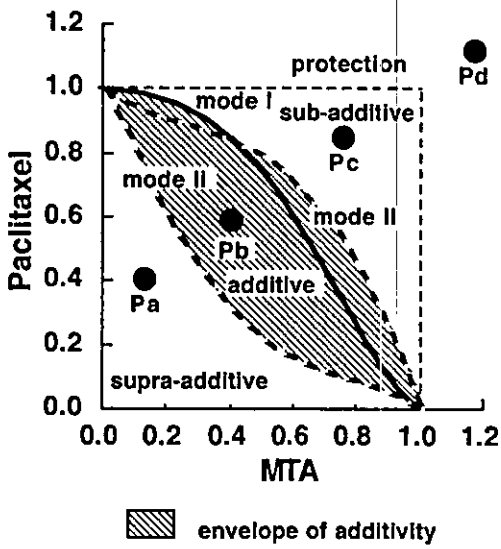

Fig. 1 Schematic representation of an isobologram (Steel and Peckham) [32]. The envelope of additivity, surrounded by mode I (*solid line*) and mode II (*dotted lines*) isobologram lines, was constructed from the dose-response curves of MTA and paclitaxel. The concentrations which produced 80% cell growth inhibition are shown as 1.0 on the ordinate and the abscissa of all isobolograms for MCF7, PA1, and WiDr cells, while the concentrations which produced 50% cell growth inhibition are shown as 1.0 on the ordinate and the abscissa of all isobolograms for A549 cells. Combined data points Pa, Pb, Pc, and Pd show supraadditive, additive, subadditive, and protective effects, respectively

A combination that gives data points to the left of the envelope of additivity (i.e., the combined effect is caused by lower doses of the two agents than is predicted) can confidently be described as supraadditive (synergistic). A combination that gives data points to the right of the envelope of additivity, but within the square or on the line of the square can be described as subadditive (i.e., the combination is superior or equal to a single agent but is less than additive). A combination that gives data points outside the square can be described as protective (i.e., the combination is inferior in cytotoxic action to a single agent). A combination with both subadditive and/or protective interactions can confidently be described as antagonistic. The Steel and Peckham isobologram is generally more strict regarding synergism and antagonism than other methods.

Data analysis

The findings were analyzed as described previously [14]. When the observed data points of the combinations mainly fell in the area of supraadditivity or in the areas of subadditivity and protection, i.e., the mean value of the observed data was smaller than that of the predicted minimum values or larger than that of the predicted maximum values, the combinations were considered to have a synergistic or antagonistic effect, respectively. To determine whether the condition of synergism (or antagonism) truly existed, a statistical analysis was performed. The Wilcoxon signed-ranks test was used for comparing the observed data with the predicted minimum (or maximum) values for additive effects, which were closest to the observed data (i.e., the data on the boundary (mode I or mode II lines) between the additive area and supraadditive area (or subadditive and protective areas). Probability (P) values <0.05 were considered significant. Combinations with $P \geq 0.05$ were regarded as indicating additive to synergistic (or additive to antagonistic) effects. All statistical analyses were performed using the Stat View 4.01 software program (Abacus Concepts, Berkeley, Calif.).

Results

The IC_{80} values of pemetrexed for a 24-h exposure against MCF7, PA1, and WiDr cells were 3.3 ± 0.4, 0.15 ± 0.02, and 0.45 ± 0.04 μM, respectively, while those of paclitaxel against MCF7, PA1, and WiDr cells were 5.9 ± 0.4, 2.5 ± 0.06, and 5.8 ± 0.06 nM, respectively. The IC_{50} values of pemetrexed and paclitaxel for a 24-h exposure against A549 cells were 2.5 ± 0.3 μM and 3.4 ± 0.3 nM, respectively.

Figure 2 shows the dose-response curves obtained from simultaneous exposure and sequential exposure to pemetrexed and paclitaxel for the MCF7 cells. The

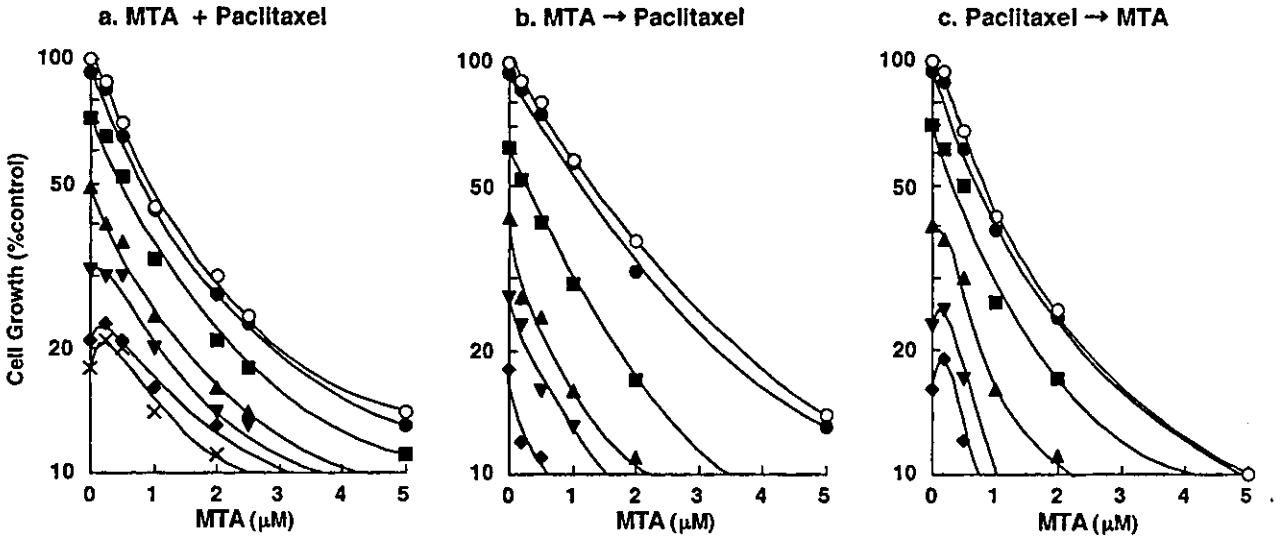

Fig. 2 Schedule dependence of the interaction between MTA and paclitaxel in MCF7 cells. Cells were exposed to (a) these two drugs simultaneously for 24 h, (b) MTA first for 24 h followed by paclitaxel for 24 h, or (c) the reverse sequence. The cell number after 5 days was measured using the MTT assay and was plotted as a percentage of the control (cells not exposed to drugs). The concentrations of MTA are shown on the abscissa. The concentrations of paclitaxel were 0 (*open circles*), 1 (*filled circles*), 2 (*filled squares*), 3 (*filled uptriangles*), 4 (*filled downtriangles*), 6 (*filled diamonds*), and 8 (*crosses*) n*M*, respectively. Data are the mean values for three independent experiments; SE was < 20%

dose-response curves were plotted on a semilog scale as a percentage of the control, the cell number of which was obtained from the samples not exposed to the drugs administered simultaneously. The pemetrexed concentrations are shown on the abscissa. Dose-response curves in which paclitaxel concentrations are shown on the abscissa could be made based on the same data (figure not shown).

Based upon the dose-response curves of pemetrexed alone and paclitaxel alone, three isoeffect curves (mode I and mode II lines) were constructed. Isobolograms at the IC_{80} and IC_{50} levels were generated based upon these dose-response curves for the combinations.

Simultaneous exposure to pemetrexed and paclitaxel for 24 h

Figure 3 shows the isobolograms of the A549, MCF7, PA1, and WiDr cells exposed to both agents simultaneously. For the A549 and PA1 cells, all or most combined data points fell in the areas of subadditivity and protection (Fig. 3a,c). The mean values of the data were larger than those of the predicted maximum data (Table 1). The differences were significant ($P < 0.05$ and $P < 0.05$), indicating antagonistic effects. For the MCF7 cells, the combined data points fell within the envelope of additivity and in the areas of subadditivity and protection (Fig. 3b; Table 1). The mean value of the data was larger than that of the predicted maximum data. The difference was not significant ($P \geq 0.05$), indicating additive/antagonistic effects. For the WiDr cells, the combined data points fell mainly within the envelope of additivity (Fig. 3d). The mean value of the data was larger than that of the predicted minimum data and smaller than that of the predicted maximum data (Table 1), indicating additive effects. A quite similar tendency was observed in the IC_{50} isobologram of the MCF7, PA1, and WiDr cells (not shown).

Sequential exposure to pemetrexed for 24 h followed by paclitaxel for 24 h

Figure 4 shows the isobolograms of the four cell lines exposed first to pemetrexed and then to paclitaxel. For the A549 and MCF7 cells, the combined data points fell in the area of supraadditivity and within the envelope of additivity (Fig. 4a,b). The mean values of the data were smaller than those of the predicted minimum data (Table 1). The differences were significant ($P < 0.05$ and $P < 0.05$), indicating synergistic effects. For the PA1 cells, the combined data points fell within the envelope of additivity (Fig. 4c), indicating additive effects (Table 1). For the WiDr cells, the combined data points fell within the envelope of additivity and in the area of supraadditivity (Fig. 4d). The mean value of the data was smaller than that of the predicted maximum data and larger than that of the predicted minimum data (Table 1), indicating additive effects. A quite similar tendency was observed in the IC_{50} isobologram of the MCF7, PA1, and WiDr cells (not shown).

Sequential exposure to paclitaxel for 24 h followed by pemetrexed for 24 h

Figure 5 shows the isobolograms of cells exposed first to paclitaxel and then to pemetrexed. For all four cell lines, all or most of the data points fell within the envelope of additivity, indicating additive effects (Table 1). A quite

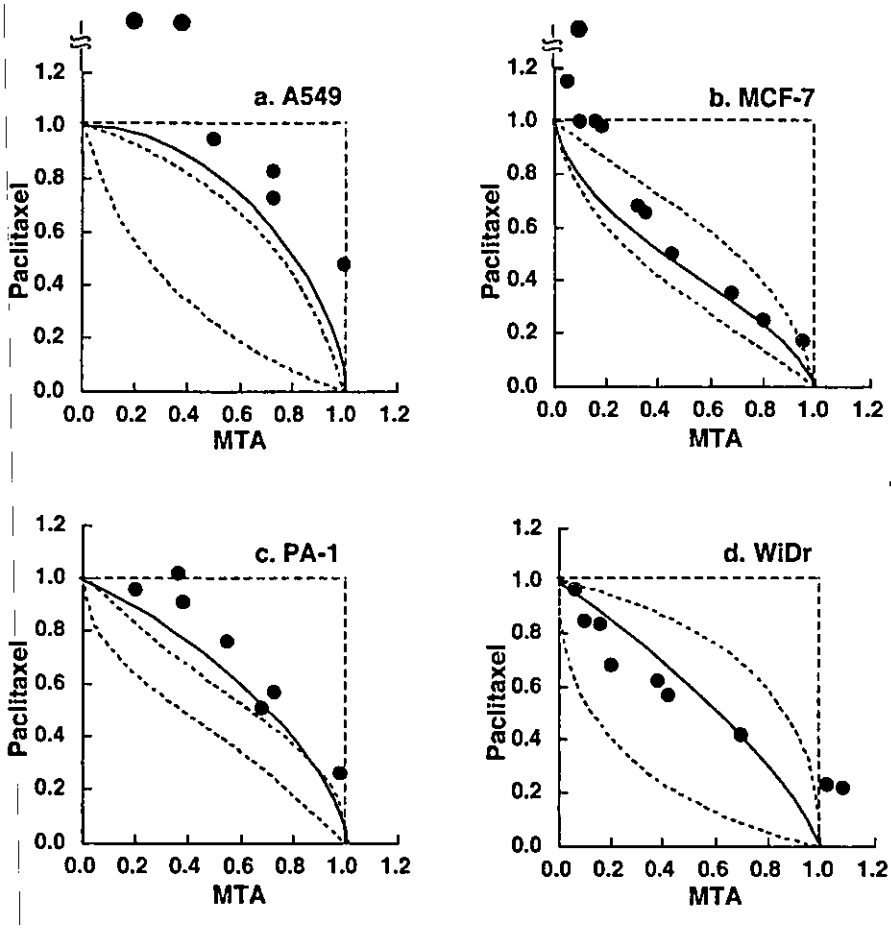

Fig. 3 Isobolograms of simultaneous exposure to MTA and paclitaxel for 24 h in (a) A549, (b) MCF7, (c) PA1, and (d) WiDr cells. For the A549, and PA1 cells, all or most combined data points fell in the areas of subadditivity and protection. For the MCF7 cells, combined data points fell within the envelope of additivity and in the areas of subadditivity and protection. For the WiDr cells, combined data points fell mainly within the envelope of additivity. Data are the mean values for at least three independent experiments; SE was < 30%

similar tendency was observed in the IC_{50} isobologram of the MCF7, PA1, and WiDr cells.

Discussion

We studied the cytotoxic activity of various schedules of pemetrexed in combination with paclitaxel in culture to investigate the optimal schedule of this combination. The analysis of the effects of drug–drug interaction was carried out using the isobologram method of Steel and Peckham [32]. Among the solid tumor cell lines studied, PA1 was most sensitive to pemetrexed, while A549 was most resistant to pemetrexed. The pemetrexed concentrations required for IC_{80} and/or IC_{50} were well within the range that can be attained in human plasma using standard dosing regimens [23].

We demonstrated that cytotoxic interactions between pemetrexed and paclitaxel were schedule-dependent and cell line-dependent. Simultaneous exposure to pemetrexed and paclitaxel showed antagonistic effects in A549 and PA1 cells, additive/antagonistic effects in MCF7

Table 1 Mean values of observed data, predicted minimum, and predicted maximum values of MTA in combination with paclitaxel at IC_{80} for MCF7, PA1 and WiDr cells and at IC_{50} for A549 cells

Schedule	Cell line	*n*	Observed data	Predicted data for an additive effect		Effect
				Minimum	Maximum	
MTA + paclitaxel	A549	6	> 0.92	0.22	0.69	Antagonism ($P < 0.05$)
	MCF7	11	0.61	0.42	0.52	Additive/antagonism
	PA1	7	0.71	0.33	0.60	Antagonism ($P < 0.05$)
	WiDr	9	0.61	0.29	0.78	Additive
MTA → paclitaxel	A549	8	0.31	0.36	0.80	Synergism ($P < 0.05$)
	MCF7	8	0.45	0.60	0.66	Synergism ($P < 0.05$)
	PA1	7	0.41	0.32	0.70	Additive
	WiDr	10	0.34	0.33	0.83	Additive
Paclitaxel → MTA	A549	6	0.78	0.31	0.82	Additive
	MCF7	8	0.58	0.44	0.66	Additive
	PA1	6	0.55	0.44	0.67	Additive
	WiDr	9	0.64	0.25	0.93	Additive

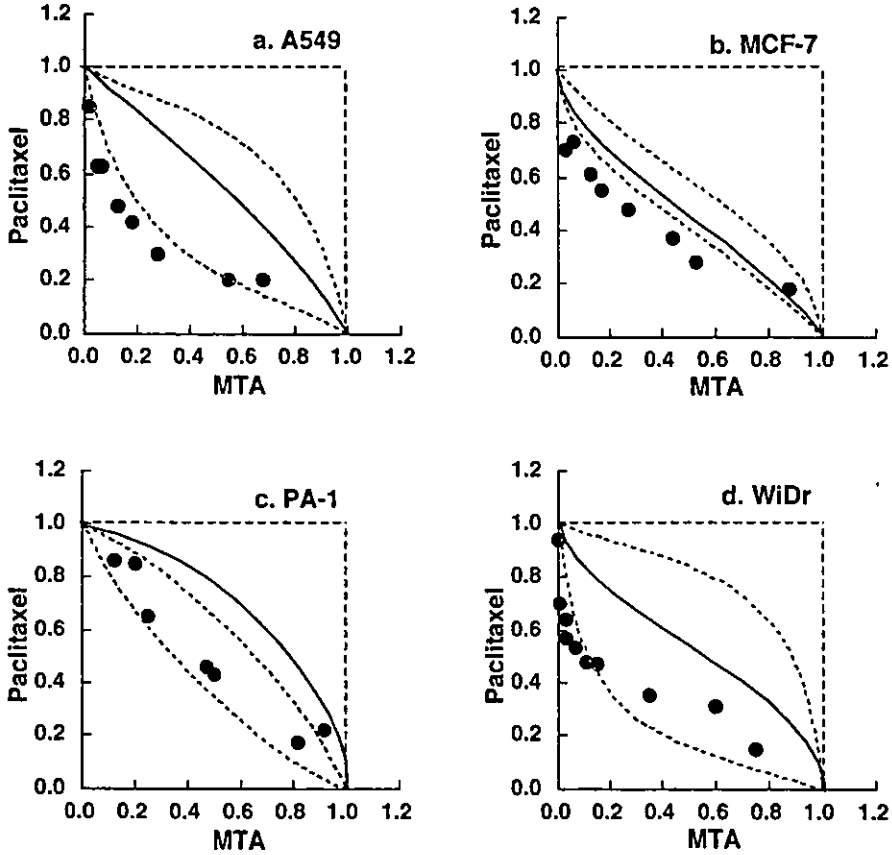

Fig. 4 Isobolograms of sequential exposure to MTA (24 h) followed by paclitaxel (24 h) in (a) A549, (b) MCF7, (c) PA1, and (d) WiDr cells. For the A549 and MCF7 cells, most data points of the combinations fell in the area of supraadditivity. For the PA1 cells, all the data points fell within the envelope of additivity. For the WiDr cells, the data points fell within the envelope of additivity and in the area of supraadditivity. Data are the mean values for at least three independent experiments; SE was < 20%

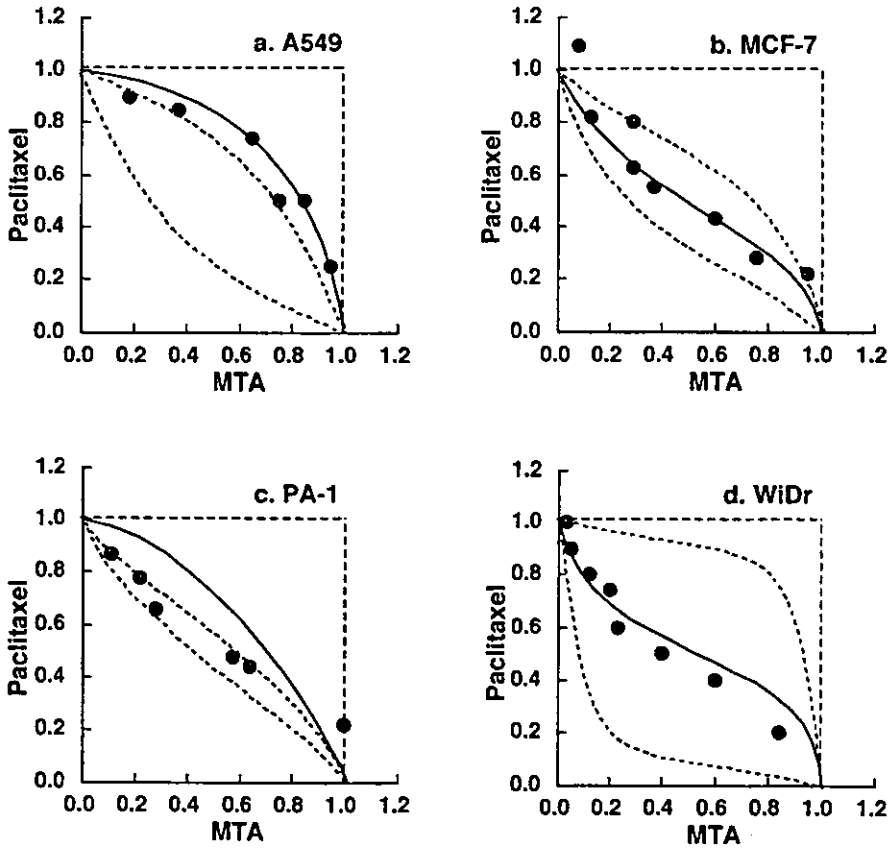

Fig. 5 Isobolograms of sequential exposure to paclitaxel (24 h) followed by MTA (24 h) in (a) A549, (b) MCF7, (c) PA1, and (d) WiDr cells. For all four cells, all or most data points of the combinations fell within the envelope of additivity. Data are the mean values for at least three independent experiments; SE was < 25%

cells and additive effects in WiDr cells. Sequential exposure to pemetrexed for 24 h followed by paclitaxel showed synergistic effects in A549 and MCF7 cells and additive effects in PA1 and WiDr cells. However, the combined data points in PA1 and WiDr cells were close to the borderlines between supraadditive and additive areas (Fig. 4), and the observed data were close to the predicted minimum values for an additive effect (Table 1). The combined data points in WiDr cells fell both in the area of supraadditivity and within the envelope of additivity (Fig. 4). Since the isobologram of Steel and Peckham is more strict for synergism and antagonism than other methods for evaluating the effects of drug combinations, simultaneous exposure to pemetrexed and paclitaxel and sequential exposure to pemetrexed followed by paclitaxel would be defined as having antagonistic and synergistic effects, respectively, using other methods.

On the other hand, sequential exposure to paclitaxel followed by pemetrexed showed additive effects in all four cell lines tested. The results of flow cytometric analysis of PA1 cells were consistent with these findings. Enhanced apoptosis was observed only in the pemetrexed–paclitaxel sequence (data not shown).

Our findings suggest that the simultaneous administration of pemetrexed and paclitaxel on the same day is convenient for clinical use but is suboptimal. The sequential administration of pemetrexed followed by paclitaxel may be the optimal schedule for these combinations. For example, administrations of pemetrexed on day 1 and paclitaxel on day 2 would be worthy of clinical investigation. Several in vitro and in vivo studies of combinations of pemetrexed with paclitaxel have been reported [28, 34, 35]. Schultz et al. observed synergistic effects when pemetrexed exposure preceded paclitaxel exposure by 24 h, while the reverse order produced only additive effects in three human cancer cells in vitro [28]. Although the detailed experimental systems are not described in the abstract, our data support their findings.

Teicher et al. studied the combination of pemetrexed and paclitaxel in vivo against EMT-6 murine mammary carcinoma using a tumor cell survival assay [34]. They observed that pemetrexed administered four times over 48 h with paclitaxel administered with the third dose of pemetrexed produced an additive or more than additive tumor response. They further studied the combination of pemetrexed and paclitaxel in human tumor xenografts [35]. Administration of pemetrexed (days 7–11, days 14–18) along with paclitaxel (days 8, 10, 12, and 15) produced greater-than-additive effects on human lung cancer H460 tumor growth delay, while that of pemetrexed (days 7–11) along with paclitaxel (days 7, 9, 11, and 13) produced additive effects on human breast cancer MX-1 tumor growth delay. Since the schedules of administration of pemetrexed with paclitaxel were quite different from ours, comparison seems difficult.

The mechanisms underlying the schedule-dependent synergism and antagonism of the combination of pemetrexed and paclitaxel are unclear. Cell cycle analysis showed that initially exposing cells to pemetrexed leads to synchronization in the S phase (data not shown). Cells in the S phase are sensitive to paclitaxel, in addition to cells in G_2/M phase [17]. This may explain the synergistic effects of sequential exposure to pemetrexed followed by paclitaxel. Simultaneous exposure to pemetrexed and paclitaxel produced antagonistic effects. Pemetrexed has a cytotoxic effect by blocking cells in the S phase [38], while paclitaxel has cytotoxic effects by blocking cells in the G_2/M phase [17, 27]. Thus, one agent might reduce the cytotoxicity of the other agent by preventing cells from entering the specific phase in which the cells are most cytotoxic to the other agent. Interestingly, we have observed similar cytotoxic interactions between methotrexate and paclitaxel [15]. Simultaneous exposure to methotrexate and paclitaxel produces antagonistic effects, while the methotrexate/paclitaxel sequence produces synergistic effects and the reverse sequence produces additive effects. These experimental data suggest that antifolates, which inhibit dihydrofolate reductase, may enhance the cytotoxic action of paclitaxel in sequential administration.

It should be noted that in vitro studies cannot evaluate toxic and pharmacokinetic interactions. Thus, in vivo studies are required to confirm whether the pemetrexed–paclitaxel sequence is optimal or not. In clinical oncology, drug interaction may result in synergism, not only in terms of efficacy but also in terms of toxic side effects. If the toxicities of the drug combinations were compared between the schedules of synergistic and antagonistic interactions at the same doses, the schedules with antagonistic interactions may produce less toxicity than the schedules with synergistic interactions. Our data showed that the drug doses required for IC_{80} or IC_{50} levels with sequential exposure to pemetrexed followed by paclitaxel are less than 70% of the drug doses required for IC_{80} or IC_{50} with simultaneous exposure to the two agents (Figs. 3 and 4). This suggests that the optimal doses for sequential administration of pemetrexed followed by paclitaxel may be lower than those for the simultaneous administration of the two agents. This is important and must be kept in mind for translating in vitro data to clinical applications, since the schedule showing antagonistic effects of the combination may be selected because of less toxicity during the first stage of clinical study.

In conclusion, our findings suggest that the cytotoxic effects of the combination of pemetrexed and paclitaxel are schedule-dependent. The optimal schedule of pemetrexed in combination with paclitaxel is the sequential administration of pemetrexed followed by paclitaxel. Although there are a number of difficulties in the translation of results from in vitro to clinical therapy, this schedule should be assessed in clinical trials for the treatment of solid tumors.

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References

1. Arbuck SG (1995) Paclitaxel: current developmental approaches of the National Cancer Institute. Semin Oncol 22:55–56
2. Britten CD, Izbicka E, Hilsenbeck S, Lawrence R, Davidson K, Cerna C, Gomez L, Rowinsky EK, Weitman S, Van Hoff DD (1999) Activity of the multitargeted antifolate MTA in the human tumor cloning assay. Cancer Chemother Pharmacol 44:105–110
3. Calvert AH, Walling JM (1998) Clinical studies with MTA. Br J Cancer 78 [Suppl 3]:35–40
4. Celio L, Buzzoni R, Longarini R, Marchiano A, Bajetta E (2002) Pemetrexed in gastric cancer: clinical experience and future perspectives. Semin Oncol 29 [Suppl 18]:63–68
5. Chen VJ, Bewley JR, Smith PG, Andis SL, Schultz RM, Iversen PW, Tonkinson JL, Shih C (2000) An assessment of the antithymine and antipurine characteristics of MTA (LY231514) in CCRF-CEM cells. Adv Enzyme Regul 40:143–154
6. Donehower RC, Rowinsky EK (1993) An overview of experience with taxol (paclitaxel) in the USA. Cancer Treat Rev 19 [Suppl C]:63–78
7. Habeck LL, Mendelsohn LG, Shih C, Taylor EC, Colman PD, Gossett LS, Leitner TA, Schultz RM, Andis SL, Moran RG (1995) Substrate specificity of mammalian folypolyglutamate synthetase for 5,10-dideazatetrahydrofolate analogs. Mol Pharmacol 48:326–333
8. Haller DG (2002) Future directions in the treatment of pancreatic cancer. Semin Oncol 29 [Suppl 20]:31–39
9. Hanauske AR, Chen V, Paoletti P, Niyikiza C (2001) Pemetrexed disodium: a novel antifolate clinically active against multiple solid tumors. Oncologist 6:363–373
10. Hochster H (2002) The role of pemetrexed in the treatment of colorectal cancer. Semin Oncol 29 [6 Suppl 18]:54–56
11. Kano Y, Ohnuma T, Okano T, Holland JF (1988) Effects of vincristine in combination with methotrexate and other antitumor agents in human acute lymphoblastic leukemia cells in culture. Cancer Res 48:351–356
12. Kano Y, Sakamoto S, Kasahara T, Akutsu M, Inoue Y, Miura Y (1991) In vitro effects of amsacrine in combination with other anticancer agents. Leuk Res 15:1059–1064
13. Kano Y, Akutsu M, Tsunoda S, Ando J, Matsui J, Suzuki K, Ikeda T, Inoue Y, Adachi K (1996) Schedule-dependent interaction between paclitaxel and 5-fluorouracil in human carcinoma cell lines in vitro. Br J Cancer 74:704–710
14. Kano Y, Akutsu M, Tsunoda S, Suzuki K, Adachi K (1998) In vitro schedule-dependent interaction between paclitaxel and SN-38 (the active metabolite of irinotecan) in human carcinoma cell lines. Cancer Chemother Pharmacol 42:91–98
15. Kano Y, Akutsu M, Tsunoda S, Furuta M, Yazawa Y, Ando J (1998) Schedule-dependent synergism and antagonism between paclitaxel and methotrexate in human carcinoma cell lines. Oncol Res 10:347–354
16. Kano Y, Akutsu M, Tsunoda S, Mano H, Sato Y, Honma Y, Furukawa Y (2001) In vitro cytotoxic effects of a tyrosine kinase inhibitor STI571 in combination with commonly used antileukemic agents. Blood 97:1999–2007
17. Lieu CH, Chang YN, Lai YK (1997) Dual cytotoxic mechanisms of submicromolar taxol on human leukemia HL-60 cells. Biochem Pharmacol 53:1587–1596
18. McDonald AC, Vasey PA, Adams L, Walling J, Woodworth JR, Abrahams T, McCarthy S, Bailey NP, Siddiqui N, Lind MJ, Calvert AH, Twelves CJ, Cassidy J, Kaye SB (1998) A phase I and pharmacokinetic study of LY231514, the multitargeted antifolate. Clin Cancer Res 4:605–610
19. O'Shaughnessy JA, Gennari A, Conte P (2002) Pemetrexed: a promising new treatment for breast cancer. Semin Oncol 29 [2 Suppl 5]:36–41
20. Paz-Ares L, Ciruelos E, Garcia-Carbonero R, Castellano D, Lopez-Martin A, Cortes-Funes H (2002) Pemetrexed in bladder, head and neck, and cervical cancers. Semin Oncol 29 [Suppl 18]:69–75
21. Paz-Ares L, Bezares S, Tabernero JM, Castellanos D, Cortes-Funes H (2003) Review of a promising new agent—pemetrexed disodium. Cancer [Suppl] 97:2056–2063
22. Raymond E, Louvet C, Tournigand C, Coudray AM, Faivre S, De Gramont A, Gespach C (2002) Pemetrexed disodium combined with oxaliplatin, SN38, or 5-fluorouracil, based on the quantitation of drug interactions in human HT29 colon cancer cells. Int J Oncol 21:361–367
23. Rinaldi DA (1999) Overview of phase I trials of multitargeted antifolate (MTA, LY231514). Semin Oncol 26 [Suppl 6]:82–88
24. Rinaldi DA, Burris HA, Dorr FA, Woodworth JR, Kuhn JG, Eckardt JR, Rodriguez G, Corso SW, Fields SM, Langley C, Clark G, Faries D, Lu P, Van Hoff DD (1995) Initial phase I evaluation of the novel thymidylate synthase inhibitor, LY231514, using the modified continual reassessment method for dose escalation. J Clin Oncol 13:2842–2850
25. Rinaldi DA, Kuhn JG, Burris HA, Dorr FA, Rodriguez G, Eckhardt SG, Jones S, Woodworth JR, Baker S (1999) A phase I evaluation of multitargeted antifolate (MTA, LY231514), administered every 21 days, utilizing the modified continual reassessment method for dose escalation. Cancer Chemother Pharmacol 44:372–380
26. Scagliotti GV, Shin DM, Kindler HL, Vasconcelles MJ, Keppler U, Manegold C, Burris H, Gatzemeier U, Blatter J, Symanowski JT, Rusthoven JJ (2003) Phase II study of pemetrexed with and without folic acid and vitamin B_{12} as front-line therapy in malignant pleural mesothelioma. J Clin Oncol 21:1556–1561
27. Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277:665–667
28. Schultz RM, Dempsey JA, Kraus LA, Schmid SM, Calvete JA, Laws AL (1999) In vitro sequence dependence for the multitargeted antifolate (MTA, LY231514) combined with other anticancer agents. Eur J Cancer 35 [Suppl 4]:S194
29. Shih C, Thornton DE (1998) Preclinical pharmacology studies and the clinical development of a novel multitargeted antifolate, MTA (LY231514). In: Jackman AL (ed) Anticancer drug development guide: antifolate drugs in cancer therapy. Humana, Totowa, p 183
30. Shih C, Chen VJ, Gossett LS, Gates SB, MacKellar WC, Habeck LL, Shackelford KA, Mendelsohn LG, Soose DJ, Patel VF, Andis SL, Bewley JR, Rayl EA, Moroson BA, Beardsley GP, Kohler W, Ratnam M, Schultz RM (1997) LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res 57:1116–1123
31. Shih C, Habeck LL, Mendelsohn LG, Chen VJ, Schultz RM (1998) Multiple folate enzyme inhibition: mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). Adv Enzyme Regul 38:135–152
32. Steel GG, Peckham MJ (1979) Exploitable mechanisms in combined radiotherapy–chemotherapy: the concept of additivity. Int J Radiat Oncol Biol Phys 5:85–91
33. Taylor EC, Kuhnt D, Shih C, Rinzel SM, Grindey GB, Barredo J, Jannatipour M, Moran RG (1992) A dideazatetrahydrofolate analogue lacking a chiral center at C-6, *N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid, is an inhibitor of thymidylate synthase. J Med Chem 35:4450–4454
34. Teicher BA, Alvarez E, Liu P, Lu K, Menon K, Dempsey J, Schultz RM (1999) MTA (LY231514) in combination treatment regimens using human tumor xenografts and the EMT-6 murine mammary carcinoma. Semin Oncol 28:55–62
35. Teicher BA, Chen V, Shih C, Menon K, Forler PA, Phares VG, Amsrud T (2000) Treatment regimens including the multitargeted antifolate LY231514 in human tumor xenografts. Clin Cancer Res 6:1016–1023

36. Tesei A, Ricotti L, De Paola F, Amadori D, Frassineti GL, Zoli W (2002) In vitro schedule-dependent interactions between the multitargeted antifolate LY231514 and gemcitabine in human colon adenocarcinoma cell lines. Clin Cancer Res 8:233–239
37. Tomek S, Emri S, Krejcy K, Manegold C (2003) Chemotherapy for malignant pleural mesothelioma: past results and recent developments. Br J Cancer 88:167–174
38. Tonkinson JL, Marder P, Andis SL, Schultz RM, Gossett LS, Shih C, Mendelsohn LG (1997) Cell cycle effects of antifolate antimetabolites: implications for cytotoxicity and cytostasis. Cancer Chemother Pharmacol 39:521–531
39. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C, Paoletti P (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 21:2636–2644

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ORIGINAL ARTICLE

A Randomized Trial of Adjuvant Chemotherapy with Uracil–Tegafur for Adenocarcinoma of the Lung

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ABSTRACT

BACKGROUND

In a previous phase 3 trial of adjuvant chemotherapy after resection of non–small-cell lung cancer, a combination of uracil and tegafur (often referred to as UFT) taken orally was shown to prolong survival. A subgroup analysis disclosed that most patients who benefited had pathological stage I adenocarcinoma.

METHODS

We randomly assigned patients with completely resected pathological stage I adenocarcinoma of the lung to receive either oral uracil–tegafur (250 mg of tegafur per square meter of body-surface area per day) for two years or no treatment. Randomization was performed with stratification according to the pathological tumor category (T1 vs. T2), sex, and age. The primary end point was overall survival.

RESULTS

From January 1994 through March 1997, 999 patients were enrolled. Twenty patients were found to be ineligible and were excluded from the analysis after randomization; 491 patients were assigned to receive uracil–tegafur and 488 were assigned to observation. The median duration of follow-up for surviving patients was 73 months. The difference in overall survival between the two groups was statistically significant in favor of the uracil–tegafur group ($P=0.04$ by a stratified log-rank test). Grade 3 toxic effects occurred in 10 of the 482 patients (2 percent) who actually received uracil–tegafur.

CONCLUSIONS

Adjuvant chemotherapy with uracil–tegafur improves survival among patients with completely resected pathological stage I adenocarcinoma of the lung.

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THE COMBINATION OF URACIL AND tegafur (also referred to as UFT) at a molar ratio of 4:1 is an oral anticancer agent with good absorption in the small intestine.[1] Tegafur is a prodrug that is gradually converted to fluorouracil in the liver by the cytochrome P-450 enzyme. Uracil enhances the serum concentration of fluorouracil by competitive inhibition of dihydropyrimidine dehydrogenase, the enzyme responsible for fluorouracil catabolism.[2] Oral uracil–tegafur generates a higher maximal plasma level of fluorouracil than the protracted intravenous injection of fluorouracil given in a dose that is equimolar to the amount of tegafur in uracil–tegafur.[3]

In patients with advanced non–small-cell lung cancer, the rate of response to treatment with uracil–tegafur ranges from 6 percent to 8 percent,[4,5] and a regimen of daily uracil–tegafur for 2 or 3 weeks plus a bolus injection of cisplatin yields a response rate of 29 to 38 percent and a median survival of 8 to 13 months.[6-8] In two trials of uracil–tegafur plus cisplatin with concurrent radiotherapy in patients with locally advanced non–small-cell lung cancer, the response rates were 80 percent[9] and 94 percent,[10] with a median survival of 16.5 months.[9] The results with uracil–tegafur plus cisplatin are similar to the results of other regimens of cisplatin-based combination chemotherapy.[11,12]

The West Japan Study Group for Lung Cancer Surgery reported that survival was significantly longer in patients assigned to adjuvant treatment with uracil–tegafur than in patients assigned to observation alone after complete resection of stage I, II, or III non–small-cell lung cancer.[13] The five-year survival rate was 64 percent in the uracil–tegafur group and 49 percent in the control group ($P=0.02$). In a subgroup analysis, there was no significant difference in overall survival between the uracil–tegafur group and the control group among patients with squamous-cell carcinoma ($P=0.24$). In contrast, patients with adenocarcinoma in the uracil–tegafur group had a significantly better survival than those in the control group ($P=0.009$).[14] In addition, most patients with adenocarcinoma had stage I disease. These results prompted us to conduct a randomized trial of uracil–tegafur as a postoperative adjuvant treatment for patients with completely resected stage I adenocarcinoma.

METHODS

PATIENTS

Enrollment began in January 1994. Eligible patients had undergone a complete surgical resection of a pathologically documented stage I (T1N0M0 or T2N0M0) adenocarcinoma of the lung (according to the 1986 classification of the American Joint Committee on Cancer).[15] Visceral pleural involvement was classified according to the rules of the Japan Lung Cancer Society,[16] and a tumor that was larger than 3 cm in diameter or a tumor of any size that was exposed on the visceral pleural surface was classified as a pathological T2 tumor. Other inclusion criteria were an age of 45 to 75 years; the absence of preoperative anticancer treatment, previous cancer, and synchronous multiple cancers; an Eastern Cooperative Oncology Group (ECOG) performance status[17] of 0, 1, or 2; a leukocyte count of at least 4000 per cubic millimeter; a platelet count of at least 100,000 per cubic millimeter; a hemoglobin level of at least 100 g per liter; serum aspartate aminotransferase and alanine aminotransferase levels that were no more than twice the upper limit of the normal range; and an absence of severe postoperative complications, such as pneumonia or empyema. Written or oral informed consent was obtained from all patients or their representatives, and the study was approved by the institutional review board of each participating center.

Confirmation of eligibility and randomization were performed by telephone or fax at a central site within 28 days after each patient's operation. All eligible patients were stratified according to age (less than 65 years vs. 65 years or older), sex, and pathological tumor category (T1 vs. T2).[18]

TREATMENT

Patients assigned to the control group were observed, with no treatment after surgery. In the treatment group, uracil–tegafur (250 mg of tegafur per square meter of body-surface area per day) in the form of 100-mg capsules (100 mg of tegafur plus 224 mg of uracil) was given orally before meals twice daily for two years, starting four weeks postoperatively. The dose was rounded up or down to the nearest 100 mg. Most patients received two capsules of uracil–tegafur (200 mg of tegafur and 448 mg of uracil) twice daily. The patients were asked at each follow-up visit whether they had taken the capsules as prescribed.

Toxic effects of uracil–tegafur were graded according to the criteria of the Japan Society of Clinical Oncology, which consist of the World Health Organization criteria with minor modifications.[19] If a grade 2 adverse reaction occurred, the dose of uracil–tegafur was reduced to 200 mg per square meter. Treatment was stopped if there was a grade 3 or higher adverse reaction, a leukocyte count of less than 3000 per cubic millimeter, a platelet count of less than 70,000 per cubic millimeter, a hemoglobin level of less than 9.5 g per deciliter, or an aspartate aminotransferase or alanine aminotransferase level that was more than three times the upper limit of the normal range.

FOLLOW-UP

A follow-up evaluation was performed every three months for the first two years after the operation and every six months thereafter. The evaluation included a physical examination, a complete blood count, blood chemical tests, screening for serum tumor markers, and chest radiography. A computed tomographic (CT) scan of the thorax and brain and either a CT scan or a sonogram of the upper abdomen were obtained every six months for the first two years after the operation and at least twice during the subsequent three years. Whenever possible, a biopsy of any new lesion suspected of being a recurrence or a second primary cancer was performed. A final diagnosis of such lesions was made by the physician in charge.

STATISTICAL ANALYSIS

The primary end point was overall survival; secondary end points were cancer-free survival and safety. All eligible patients were included in the analysis of overall survival and cancer-free survival, and all patients who were given uracil–tegafur were included in the safety assessment.

The sample size was calculated by the method of Schoenfeld and Richter[20] according to the following assumptions: a five-year survival rate of 70 percent in the no-treatment group, a hazard ratio for death of 0.67 in the uracil–tegafur group, a two-year accrual period, a five-year follow-up, a one-sided significance level of 0.05, and a statistical power of 80 percent. Since these calculations resulted in a sample size of 518 patients, the sample size was determined to be 600, with an allowance of about 15 percent for ineligible patients or patients who were lost to follow-up. In May 1995, the sample size was expanded to 984 patients after it became clear that the five-year survival rate for those in the control group was better than expected. The newly adopted five-year survival rate was 83 percent, and the accrual period was extended to three years. A committee for efficacy and safety provided independent monitoring of the study. Haybittle–Peto horizontal boundaries,[21] with a criterion of $P<0.001$, were used in the interim analyses conducted to determine whether the study should be terminated early.

Overall survival was defined as the time from surgery until death from any cause, and cancer-free survival was defined as the time from surgery until the appearance of the first recurrence of cancer, a second cancer, or death from any cause. Survival was estimated by the Kaplan–Meier method, and any differences in survival were evaluated with a stratified log-rank test. Multivariable analyses with the Cox proportional-hazards model were used to estimate the simultaneous effects of prognostic factors on survival.[22] Interactions with prognostic factors were also examined with the Cox proportional-hazards model. The SAS statistical software package (version 6.09, SAS Institute) was used for all calculations. Differences were considered to be statistically significant when the P value was 0.05 or less. All statistical tests were two-sided.

The protocol committee of the Japan Lung Cancer Research Group designed the study. Taiho Pharmaceutical Company collected and analyzed the data, and the authors interpreted the data and wrote the report. The authors had access to the primary data.

RESULTS

CHARACTERISTICS OF THE PATIENTS

From January 1994 through March 1997, 999 patients were enrolled and randomly assigned to receive uracil–tegafur (498 patients) or no treatment (501 patients). Seven patients in the uracil–tegafur group and 13 patients in the control group were ineligible for the following reasons: pathological N1 or M1 disease in 7 patients, histologic findings other than adenocarcinoma in 6, no laboratory data at registration in 2, and miscellaneous reasons in 5. Therefore, there were 491 eligible patients in the uracil–tegafur group and 488 in the control group. Table 1 lists the base-line clinical characteristics of the two groups, which did not differ significantly. All but one patient in each group underwent lobectomy.

Table 1. Base-Line Characteristics of the Patients.

Characteristic	Uracil–Tegafur Group (N=491)	Control Group (N=488)
Age		
Mean (yr)	62	62
Range (yr)	45–75	45–75
<65 yr (no.)	274	275
≥65 yr (no.)	217	213
Female sex (no.)	253	249
ECOG performance status (no.)*		
0	376	369
1	105	113
2	10	6
Pathological tumor stage (no.)		
T1	362	354
T2	129	134
Invasion of pleura (no.)†		
0	340	346
1	120	114
2	29	28
Unknown	2	0
Tumor size (no.)		
≤2 cm	208	204
>2 to ≤3 cm	174	170
>3 cm	109	114
Location of the tumor (no.)		
Right upper lobe	182	189
Right middle lobe	41	34
Right lower lobe	102	87
Right lobes	2	2
Left upper lobe	107	114
Left lower lobe	54	60
Left lobes	3	2
Type of surgery (no.)		
Lobectomy	490	487
Pneumonectomy	1	1

* ECOG denotes Eastern Cooperative Oncology Group. Higher performance-status numbers indicate greater impairment.

† 0 indicates a tumor with no pleural involvement or a tumor that reaches the visceral pleura but does not extend beyond the elastic layer, 1 a tumor that extends beyond the elastic layer of the visceral pleura but is not exposed on the pleural surface, and 2 a tumor that is exposed on the pleural surface but does not involve the parietal pleura.

ADVERSE REACTIONS AND COMPLIANCE

Of the 498 patients originally assigned to the uracil–tegafur group, 482 actually received uracil–tegafur. Few severe adverse reactions were associated with uracil–tegafur. A grade 3 adverse reaction developed in 10 of 482 patients (2 percent), and no grade 4 adverse reactions occurred (Table 2).

Compliance with instructions to take uracil–tegafur was calculated on the basis of the number of patients who actually took uracil–tegafur and the number of patients who were assigned to it, excluding those with a recurrence or second cancer and those who died. The rate of compliance was 80 percent (95 percent confidence interval, 77 to 84 percent) at 6 months, 74 percent (95 percent confidence interval, 70 to 78 percent) at 12 months, 69 percent (95 percent confidence interval, 65 to 73 percent) at 18 months, and 61 percent (95 percent confidence interval, 57 to 66 percent) at 24 months. The main reasons for discontinuation of uracil–tegafur were an adverse reaction (in 123 patients), the patient's decision (52 patients), and the doctor's judgment (34 patients).

OVERALL SURVIVAL

The median follow-up among surviving patients was 72 months in the uracil–tegafur group and 73 months in the control group. Data were censored for 426 patients in the uracil–tegafur group and 399 in the control group. At the last follow-up visit, 65 patients in the uracil–tegafur group and 89 in the control group had died, and the overall survival rates in the two groups differed significantly on the basis of the stratified log-rank test (Fig. 1A). The five-year overall survival rate was 88 percent (95 percent confidence interval, 85 to 91 percent) in the uracil–tegafur group and 85 percent (95 percent confidence interval, 82 to 89 percent) in the control group. When the survival analysis was performed with the inclusion of all 999 randomized patients, the result did not change (P=0.047).

The predetermined covariates were age (<65 years vs. ≥65 years), sex, ECOG performance status (0 vs. 1 or 2), pathological T status (T1 vs. T2), and the assigned treatment. The covariates were selected according to multivariate analysis with the use of a stepwise procedure. All P values were less than 0.05. The selected covariates were as follows: age (hazard ratio for patients ≥65 years, 2.02; 95 percent confidence interval, 1.46 to 2.80; P<0.001), sex (hazard ratio for women, 0.66; 95 percent confidence interval, 0.48 to 0.91; P=0.01), T category (hazard ratio for T2, 1.95; 95 percent confidence interval, 1.41 to 2.69; P<0.001), and treatment group (hazard ratio for the uracil–tegafur group, 0.72; 95 percent confidence interval, 0.53 to 1.00; P=0.05).

Table 2. Adverse Reactions to Uracil–Tegafur.

Adverse Reaction	Grade of Toxicity*			
	1	2	3	4
	% of patients			
Leukopenia	2	1	0	0
Thrombocytopenia	<1	0	0	0
Anemia	1	<1	0	0
Increase in bilirubin	1	<1	0	0
Increase in aspartate aminotransferase	6	2	<1	0
Increase in alanine aminotransferase	6	2	0	0
Increase in alkaline phosphatase	2	<1	0	0
Anorexia	9	8	1	0
Nausea or vomiting	10	3	1	0
Diarrhea	2	1	<1	0
Alopecia	<1	0	0	0

* Toxicity was graded according to criteria of the Japan Society of Clinical Oncology. Grades range from 1 to 4, with a higher grade indicating a more severe reaction.

We also evaluated interactions between the four prognostic factors (sex, age, pathological tumor category, and size of the tumor) (Fig. 2) and the treatment. We included tumor size in the analysis because the tumor category is determined mainly by the maximal diameter of the primary tumor. As Figure 2 shows, there were significant interactions between the tumor category and size of the tumor and the treatment.

The survival rate among patients with T2 disease in the uracil–tegafur group was significantly higher than that in the control group, whereas among patients with T1 disease, there was no significant difference in survival between the uracil–tegafur and control groups. The five-year survival rate among patients with T2 disease was 85 percent (95 percent confidence interval, 79 to 91 percent) in the uracil–tegafur group and 74 percent (95 percent confidence interval, 66 to 81 percent) in the control group (Fig. 1B). The difference in overall survival between the two groups was statistically significant (P=0.005 by the log-rank test). The five-year survival rate among patients with T1 disease was 89 percent in the uracil–tegafur group and 90 percent in the control group (Fig. 1C). In the subgroups of patients with a tumor that was less than 2 cm in diameter, 2 to 3 cm, and greater than 3 cm, the five-year survival rate was 89 percent, 89 percent, and 85 per-

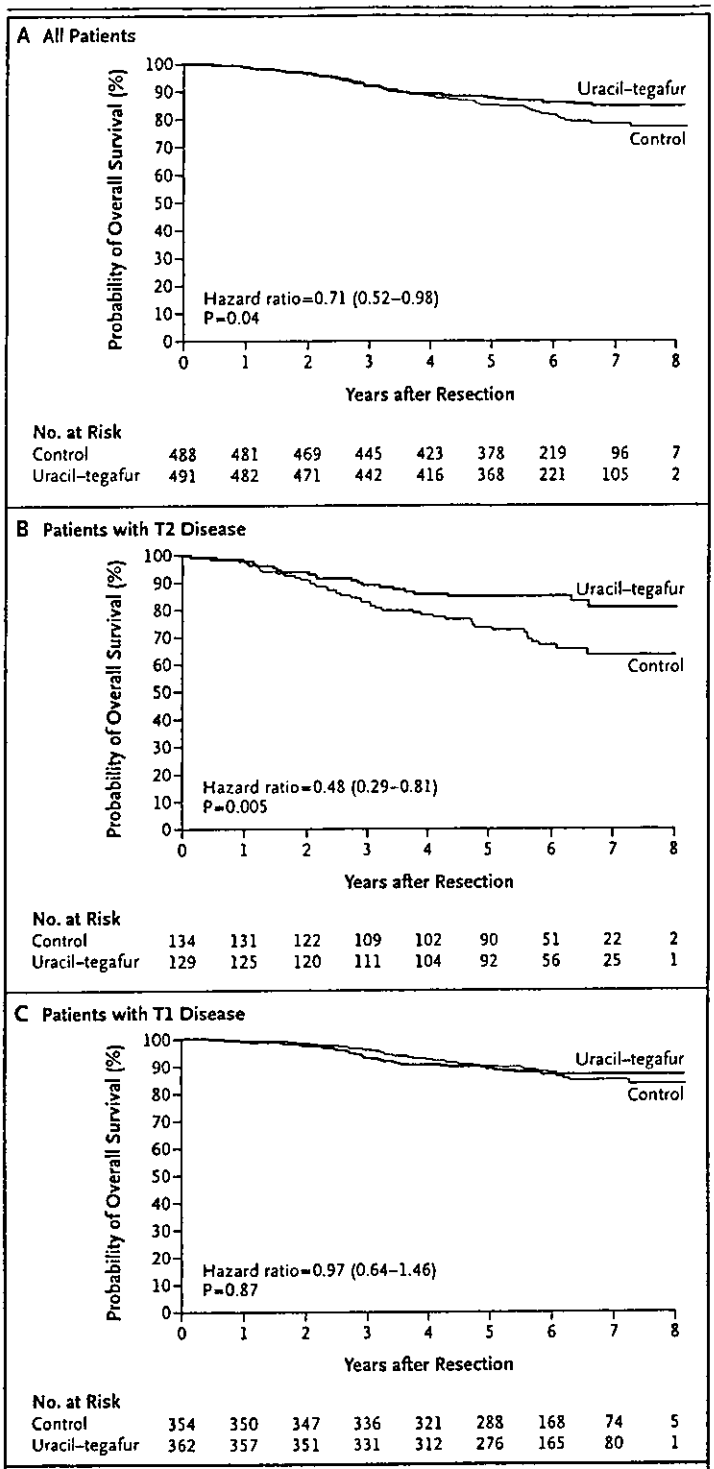

Figure 1. Overall Survival among All 979 Eligible Patients (Panel A), 263 Patients with T2 Disease (Panel B), and 716 Patients with T1 Disease (Panel C) Who Were Randomly Assigned to the Uracil–Tegafur Group and the Control Group.

The hazard ratios indicate the risk of death in the uracil–tegafur group as compared with the control group; 95 percent confidence intervals are shown in parentheses. P values were calculated with the use of the stratified log-rank test.

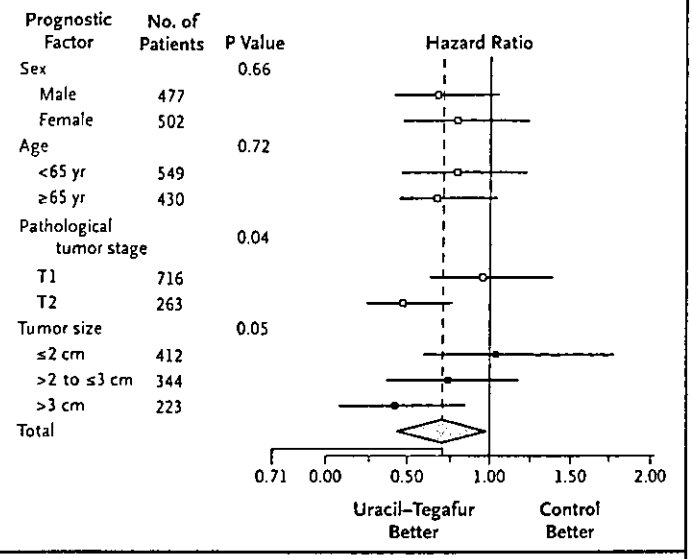

Figure 2. Hazard Ratios for Death in Patients in the Uracil–Tegafur Group as Compared with the Control Group, According to Four Prognostic Factors.

Each square represents the estimated treatment effect, the horizontal lines represent the 95 percent confidence intervals, and the diamond corresponds to the 95 percent confidence intervals for the entire group of patients. The P value for the tumor size is for the comparison of patients who had tumors that were 2 cm or less in diameter with patients who had tumors that were more than 3 cm.

cent, respectively, in the uracil–tegafur group and 91 percent, 86 percent, and 74 percent, respectively, in the control group.

PATTERN OF FAILURE AND CANCER-FREE SURVIVAL

A recurrence or a second primary cancer as the first treatment failure after surgery was documented in 23 percent of the uracil–tegafur group and 26 percent of the control group (Table 3). Among the 716 patients with T1 disease, recurrence or a second primary cancer was observed in 69 of 362 patients (19 percent) in the uracil–tegafur group and 76 of 354 patients (21 percent) in the control group; among the 263 patients with T2 disease, 42 of 129 patients (33 percent) in the uracil–tegafur group and 53 of 134 patients (40 percent) in the control group had recurrence or a second primary cancer as the first treatment failure. On the basis of a Kaplan–Meier analysis, the difference in cancer-free survival between the two groups was not statistically significant (P=0.25 by the stratified log-rank test). The survival of patients after the diagnosis of a recurrence or a second primary cancer did not differ significantly between the groups (P=0.14 by the log-rank test): the one-year and two-year survival rates after diagnosis were 65 percent and 50 percent, respectively, in the uracil–tegafur group and 65 percent and 42 percent, respectively, in the control group.

DISCUSSION

The Japanese Association for Chest Surgery and Japan Lung Cancer Society recently reported the long-term survival rate of 7408 patients with lung cancer who had undergone a surgical resection in 1994, the year that our trial started.[23] The main histologic types were adenocarcinoma (in 56 percent of the patients) and squamous-cell carcinoma (in 33 percent). Among patients with pathological stages T1N0M0 and T2N0M0, the five-year survival rates were 79 percent and 60 percent, respectively. In our study of adenocarcinoma, the five-year survival rate in the control group was 90 percent among patients with T1N0M0 disease and 74 percent among those with T2N0M0 disease. Although the figures in the two studies cannot be directly compared, owing to different histologic patterns and times when the data were collected, the excellent five-year survival rate for the control patients in our study[24,25] indicates that our collaborative group has made improvements in the quality of the surgical treatment and the accuracy of surgical staging.

Our study shows that adjuvant chemotherapy with uracil–tegafur has a beneficial effect on the survival of patients with resected stage I adenocarcinoma of the lung. This benefit, however, was not observed in patients with T1N0 disease. In the past few years, the number of patients in whom small adenocarcinomas have been discovered has increased owing to the increased use of computed tomography. In our study, 412 of 979 patients (42 percent) had an adenocarcinoma that was less than 2 cm in diameter. Adenocarcinomas of this size often include bronchoalveolar carcinoma, which is unlikely to recur after resection.[26] Therefore, a small adenocarcinoma usually has a very good prognosis[26,27]: in our study, the five-year survival rate of patients with tumors that were 2 cm or less in diameter was 91 percent. For this reason, we believe that patients with small tumors should be excluded from adjuvant trials unless a subgroup with a poor prognosis is identified.

In contrast, treatment with uracil–tegafur tended to improve the survival rate among patients with a tumor that was 2 to 3 cm in diameter and provided

a definitive survival benefit for patients with a tumor that was more than 3 cm in diameter. These findings indicate that the effect of uracil–tegafur may be related to certain biologic factors. In a retrospective study, Tanaka et al.[28] found that the prognosis was good for patients with non–small-cell lung cancer characterized by a high apoptotic index and no aberrant expression of p53 who received postoperative uracil–tegafur.

Patient compliance is usually a problem in trials of adjuvant chemotherapy. In trials of cisplatin-based chemotherapy, which was scheduled to be administered in three or four cycles postoperatively, only 50 to 70 percent of the planned treatment was given.[29-32] In our trial, we planned to give uracil–tegafur daily for two years. However, only 61 percent of patients assigned to the treatment completed the two-year course. The main reasons for discontinuing uracil–tegafur were adverse reactions (which were infrequent and usually mild) and the patient's decision, which suggests that compliance in trials of adjuvant chemotherapy may not be related to the severity of adverse events.

The main difference between trials of cisplatin-based adjuvant chemotherapy and trials of adjuvant chemotherapy with uracil–tegafur is the duration of the treatment. The cisplatin-based regimens entail three or four cycles (9 to 16 weeks) of chemotherapy,[29-32] whereas uracil–tegafur is taken daily for 1 or 2 years.[13,33-36] Fluorouracil is not a dose-dependent drug but a time-dependent agent. Therefore, a daily regimen of uracil–tegafur is an effective way of maintaining the blood level of fluorouracil. In addition, uracil–tegafur and its metabolites have an inhibitory effect on tumor angiogenesis in mice.[37] If this effect occurs in humans, then the daily, long-term administration of uracil–tegafur may be beneficial.

So far, six randomized trials,[13,33-36] including the present one, have been conducted that compare surgery alone with adjuvant chemotherapy with uracil–tegafur. Among them, three trials have shown a survival benefit from treatment with uracil–tegafur.[13,34] A meta-analysis of those six trials showed that adjuvant chemotherapy with uracil–tegafur improved the overall survival (hazard ratio for death, 0.77; 95 percent confidence interval, 0.63 to 0.94; P=0.01).[38] It is unclear whether patients with stage II or stage III disease benefit from treatment with uracil–tegafur and whether treatment for one year is equivalent to treatment for two years. However, our study indicates that patients with completely resected stage I disease, especially T2N0 adenocarcinoma, will benefit from adjuvant chemotherapy with uracil–tegafur.

Table 3. Pattern of Treatment Failure.

Pattern	Uracil–Tegafur Group (N=491)	Control Group (N=488)
	no. of patients (%)	
Intrathoracic only		
Local recurrence	17	8
Pulmonary metastases	36	38
Local recurrence plus pulmonary metastases	3	12
Second cancer	11	11
Extrathoracic only		
Recurrence	23	33
Second cancer	14	18
Intrathoracic plus extrathoracic recurrence	7	9
Total	111 (22.6)	129 (26.4)

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APPENDIX

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REFERENCES

1. Fujii S, Kitano S, Ikenaka K, Shirasaka T. Effect of coadministration of uracil or cytosine on the anti-tumor activity of clinical doses of 1-(2-tetrahydrofuryl)-5-fluorouracil and level of 5-fluorouracil in rodents. Gann 1979;70:209-14.
2. Ikenaka K, Shirasaka T, Kitano S, Fujii S. Effect of uracil on metabolism of 5-fluorouracil in vitro. Gann 1979;70:353-9.
3. Ho DH, Pazdur R, Covington WP, et al. Comparison of 5-fluorouracil pharmacokinetics in patients receiving continuous 5-fluorouracil infusion and oral uracil plus N_1-(2'-tetrahydrofuryl)-5-fluorouracil. Clin Cancer Res 1998;4:2085-8.
4. Shimizu E, Kimura K, Sone S, et al. A phase II study of UFT in non-small cell lung cancer. Gan To Kagaku Ryoho 1986; 13:2970-3. (In Japanese.)
5. Keicho N, Saijo N, Shinkai T, et al. Phase II study of UFT in patients with advanced non-small cell lung cancer. Jpn J Clin Oncol 1986;16:143-6.
6. Ichinose Y, Takanashi N, Yano T, et al. A phase II trial of oral tegafur and uracil plus cisplatin in patients with inoperable non-small cell lung cancer. Cancer 1995;75: 2677-80.
7. Ichinose Y, Yoshimori K, Yoneda S, Kuba M, Kudoh S, Niitani H. UFT plus cisplatin combination chemotherapy in the treatment of patients with advanced non-small cell lung carcinoma: a multiinstitutional phase II trial. Cancer 2000;88:318-23.
8. Saito J, Nakai Y, Saijo Y, et al. A phase II trial of oral UFT plus cisplatin (CDDP) in patients with non-small cell lung cancer (NSCLC). Lung Cancer 2001;31:285-93.
9. Ichinose Y, Nakai Y, Kudoh S, et al. UFT plus cisplatin with concurrent radiotherapy for locally advanced non-small-cell lung cancer (NSCLC): a multiinstitutional phase II trial. Prog Proc Am Soc Clin Oncol 2002; 21:321a. abstract.
10. Ichinose Y, Yano T, Asoh H, et al. UFT plus cisplatin with concurrent radiotherapy for locally advanced non-small-cell lung cancer. Oncology (Huntingt) 1999;13:Suppl 3:98-101.
11. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non–small-cell lung cancer. N Engl J Med 2002;346:92-8.
12. Vokes EE, Herndon JE II, Crawford J, et al. Randomized phase II study of cisplatin with gemcitabine or paclitaxel or vinorelbine as induction chemotherapy followed by concomitant chemoradiotherapy for stage IIIB non-small-cell lung cancer: Cancer and Leukemia Group B study 9431. J Clin Oncol 2002;20:4191-8.
13. Wada H, Hitomi S, Teramatsu T. Adjuvant chemotherapy after complete resection in non-small-cell lung cancer. J Clin Oncol 1996;14:1048-54.
14. Okimoto N, Soejima R, Teramatsu T. A randomized controlled postoperative adjuvant chemotherapy trial of CDDP+VDS+UFT and UFT alone in comparison with operation only for non-small cell lung carcinomas (second study). Jpn J Lung Cancer 1996;36: 863-71. (In Japanese.)
15. Mountain CF. A new international staging system for lung cancer. Chest 1986;89: Suppl:225s-233s.
16. The Japan Lung Cancer Society. General rule for clinical and pathological record of lung cancer. 3rd ed. Tokyo, Japan: Kanehara, 1987.
17. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.
18. Hamajima N, Japan Lung Cancer Research Group. Registration for a large-scale randomized controlled trial of postsurgical adjuvant chemotherapy for lung cancer in Japan. Int J Clin Oncol 1999;4:133-7.
19. WHO handbook for reporting results of cancer treatment. Geneva: World Health Organization, 1979:14-21. (Offset publication no. 48.)
20. Schoenfeld DA, Richter JR. Nomograms for calculating the number of patients needed for a clinical trial with survival as an endpoint. Biometrics 1982;38:163-70.
21. Haybittle JL. Repeated assessment of results in clinical trials of cancer treatment. Br J Radiol 1971;44:793-7.
22. Cox DR. Regression models and life-tables. J R Stat Soc [B] 1972;34:187-220.
23. Japanese Joint Committee of Lung Cancer Registry, Shirakusa T, Kobayashi K. Lung cancer in Japan: analysis of lung cancer registry for resected cases in 1994. Jpn J Lung Cancer 2002;42:555-66. (In Japanese.)
24. Breathnach OS, Kwiatkowski DJ, Finkelstein DM, et al. Bronchioloalveolar carcinoma of the lung: recurrences and survival in patients with stage I disease. J Thorac Cardiovasc Surg 2001;121:42-7.
25. Myrdal G, Lambe M, Gustafsson G, Nilsson K, Stahle E. Survival in primary lung cancer potentially cured by operation: influence of tumor stage and clinical characteristics. Ann Thorac Surg 2003;75:356-63.
26. Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung: histologic characteristics and prognosis. Cancer 1995;75:2844-52.
27. Kodama K, Higashiyama M, Yokouchi H, et al. Prognostic value of ground-glass opacity found in small lung adenocarcinoma on high-resolution CT scanning. Lung Cancer 2001;33:17-25.
28. Tanaka F, Otake Y, Yanagihara K, et al. Apoptosis and p53 status predict the efficacy of postoperative administration of UFT in non-small cell lung cancer. Br J Cancer 2001;84:263-9.
29. Feld R, Rubinstein L, Thomas PA, Lung Cancer Study Group. Adjuvant chemotherapy with cyclophosphamide, doxorubicin, and cisplatin in patients with completely resected stage I non-small-cell lung cancer. J Natl Cancer Inst 1993;85:299-306.
30. Ohta M, Tsuchiya R, Shimoyama M, et al. Adjuvant chemotherapy for completely resected stage III non-small-cell lung cancer: results of a randomized prospective study. J Thorac Cardiovasc Surg 1993;106:703-8.
31. Ichinose Y, Tada H, Koike T, et al. A randomized phase III trial of postoperative adjuvant chemotherapy in patients with completely resected stage IIIa-N2 non-small cell lung cancer: Japan Clinical Oncology Group (JCOG 9304) trial. Prog Proc Am Soc Clin Oncol 2001;20:311a. abstract.
32. Scagliotti GV, Fossati R, Torri V, et al. Randomized study of adjuvant chemotherapy for completely resected stage I, II, or IIIA non-small-cell lung cancer. J Natl Cancer Inst 2003;95:1453-61.

33. Tanaka F, Wada H, West Japan Study Group for Lung Cancer Surgery. Postoperative oral administration of UFT for completely resected pathologic stage I non-small cell lung cancer: the West Japan Study Group for Lung Cancer Surgery (WJSG), the 4th study. Prog Proc Eur Cancer Conf 2001; 37:S29. abstract.
34. Tada H, Yasumitu T, Iuchi K, Taki T, Kodama K, Mori T. Randomized study of adjuvant chemotherapy for completely resected non-small cell lung cancer: lack of prognostic significance in DNA ploidy pattern at adjuvant setting. Prog Proc Am Soc Clin Oncol 2002;21:313a. abstract.
35. Endo C, Saito Y, Iwanami H, et al. A randomized trial of postoperative UFT therapy in p stage I, II non-small cell lung cancer. Lung Cancer 2003;40:181-6.
36. Imaizumi M. A randomized trial of postoperative adjuvant chemotherapy for p-stage I non-small cell lung cancer (4th cooperative study). Prog Proc World Conf Lung Cancer 2003;41:S54. abstract.
37. Yonekura K, Basaki Y, Chikahisa L, et al. UFT and its metabolites inhibit the angiogenesis induced by murine renal cell carcinoma, as determined by a dorsal air sac assay in mice. Clin Cancer Res 1999;5:2185-91.
38. Hamada C, Ohta M, Wada H, et al. Efficacy of oral UFT for adjuvant chemotherapy after complete resection of non-small cell lung cancer: meta-analysis of six randomized trials in 2003 patients. Prog Proc Eur Cancer Conf 2003;39:S231. abstract.

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Treatment of Peripheral Early Stage Lung Cancer

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Editorial

Treatment of Peripheral Early Stage Lung Cancer

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Introduction

Not only is the incidence of lung cancer increasing around the world, this disease has become the leading cause of cancer death. Since lung cancer kills 85% to 90% of its victims, it is recognized as one of the most difficult to cure diseases. Although the therapeutic results are quite unsatisfactory as a whole, earlier stages of lung cancer, stages IA and IB show better therapeutic results (Table 1).[1] To improve the therapeutic results of lung cancer, efforts for early detection and treatment are essential. In our institution, the 5-year survival rate has gradually improved over the past five decades. These results could be due to improvement of therapeutic procedures including surgery, chemotherapy, radiotherapy, laser therapy and immunotherapy. Furthermore, the improvement of survival may be partially due to lung cancer mass screening made by the Health Insurance Act of 1987.

Lung cancer mass screening by chest computed tomography (CT) was begun in Japan 10 years ago and now is becoming subsequently used in the United States and Europe. Since large numbers of peripheral tiny lung shadows were detected in many of the CT screening pilot trials,[2,3] it is important to establish an internationally accepted definition of peripheral type early stage lung cancer.

In this editorial the authors describe the present status and prospects for the treatment of early stage lung cancer.

The Criteria of Early Stage Lung Cancer

Since there are no authorized international criteria of early stage lung cancer, establishment of criteria is urgently required. According to the location of the tumor, early stage lung cancers are classified into two categories; central type and peripheral type.

In Japan, the criteria of early stage lung cancer were first proposed about 30 years ago, in 1975. Peripheral type early stage lung cancer was defined as a tumor located in an airway more peripheral than subsegmental bronchi, and the longest dimension of the tumor should be 2 cm or less and with no recognized lymph node and distant metastases. In central type early stage lung cancer, the tumor should be located in a segmental bronchus or more proximal airway, and the depth of tumor invasion should be limited to within the bronchial wall with no lymph node or distant metastases. These criteria of central type early stage lung cancer were first defined pathologically in a resected lung by Ikeda in a study supported by the Ministry of Health and Welfare in Japan. Now we have criteria of endoscopically diagnosed central type early stage lung cancer defined by the Japan Lung Cancer Society.[4]

Therapeutic Guidelines of Early Stage Lung Cancer

In Japan, the therapeutic guidelines of lung cancer established on Evidence-based Medicine were made with the support of the Ministry of Health, Labor and Welfare in 2002. In these guidelines, surgical resection and PDT are recommended for treatment of central type early stage lung cancer.[5]

The Possibility of Limited Resection by Video-assisted Thoracoscopic Surgery (VATS)

The standard therapeutic procedure for peripheral type early stage lung cancer is believed to be lobectomy with mediastinal lymph node dissection. However the question was raised whether lobectomy is really needed for tiny tumors, particularly those less than 1 cm in greatest

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Table 1. Survival rates according to pathologic stages (n=7,047)

p-stage	n	1 year	2 year	3 year	4 year	5 year
IA	2,142	96.5	92.8	87.9	82.7	79.2
IB	1,488	90.2	80.3	72.4	65.6	60.1
IIA	261	90.7	78.6	68.4	62.9	58.6
IIB	785	81.3	64.5	52.7	47.6	42.2
IIIA	1,337	74.7	53.8	40.3	32.6	28.4
IIIB	759	64.6	40.2	28.4	22.5	20
IV	275	60.3	39.4	29.9	22.5	19.3

n: numbers of patients with lung cancer

dimension. There are several reports on limited resection of small lung cancer.[6,7] Some of these results showed satisfactory 5-year survival rates. Clinical trials to clarify the possibility of limited resection are needed for particularly small lung cancers showing ground glass opacity (GGO), or ground glass attenuation (GGA) . Most of these lesions showed no lymph node metastases, and a 100% 5-year survival was obtained in such cases who underwent resection. A multi-center clinical trial sponsored by the Japan Clinical Oncology Group (JCOG) just started to examine the suitability of limited resection for peripheral small lung cancer. Wedge resection of small lung cancer by VATS without lymph node dissection is one type of the minimally invasive surgery. If some types of lung cancer could be shown to be resected by VATS without any increase of local recurrence, this method could become a future standard treatment for peripheral small lung cancer.

The Rate of Lymph Node Metastasis of Peripheral Small Nodular Cancer

In the past five years, 783 patients with lung cancer underwent surgery in our institution. Among them there were 150 patients with peripheral nodules less than 2 cm in diameter, including 135 adenocarcinomas. Lobectomy was performed in 93 cases and limited resection was performed in 42 cases. The pathological prognostic factors were investigated for the future selection of surgical procedures in the peripheral small nodules. Of cases less than 1 cm, 97.5% of cases showed no lymph node involvement, however even in such tiny tumors 2.5% of them already showed N2 disease. In the cases between 1 and 1.5 cm, 91.9% of cases showed no metastasis, however 8.1% showed either N1 or N2 involvements. In the cases between 1.5 and 2 cm, lymph node involvement was recognized in 12%. Therefore it seems that the tumor size does not have a large correlation with lymph node involvement.

According to Noguchi's classification,[8] bronchioloalveolar cell carcinoma showing findings of GGO on CT images did not have any nodal metastases.[9] The CT images of our cases were classified into four categories according to the percentages of areas of GGO findings in relation to the entire tumor; 100% GGO, between 50% and 100%, less than 50% and 0% GGO findings. According to these criteria, 16 cases consisted of GGO in 100% of the tumor area and 21 cases consisted of between 50% and 100% GGO. These two groups showed no lymph node metastases. Furthermore, in cases with GGO findings consisting of less than 50% or 0% of the lesion, cases with a tumor size of less than 1 cm showed no lymph node metastasis. However, two cases with a tumor size more than 1 cm had nodal metastases. In the cases with 0% GGO, the presence of lymph node metastases was not related to the sizes of the tumor. The overall 5-year survival rate in adenocarcinoma 2 cm or less in tumor size was 93.3%.

The survival curves according to the postoperative stage showed a 98.1% 5-year survival rate in stage IA, 54.7% in stage IIIA and no 5-year survivals in stages IIA and IV. Since the number is small in stages IIA and IV, it is necessary to increase the number for accurate evaluation. In the survival curves according to the tumor size, tumors less than 1 cm showed a 100% 5-year survival rate. In tumors between 1 and 1.5 cm the survival rate was 86.5%, and in cases between 1.5 and 2 cm, the 5-year survival rate was 92.4%. On the survival curves according to area of GGO finding, the cases consisting of more than 50% GGO showed 100% 5-year survival rate and the cases consisting of less than 50% GGO had 91.1% 5-year survival rate. From these data it seems that the proportion of GGO in the tumor may be related to prognosis. The survival rate was 100% in cases of limited operation and 91.5% in lobectomy cases. The better result of limited resection than lobectomy might be due to selection bias.