Cancer Chemother Pharmacol (1992) 29: 185–189

Cancer Chemotherapy and Pharmacology

In vitro succinate dehydrogenase chemosensitivity of gastric carcinoma – relationship to DNA content

A. Saito, D. Korenaga, Y. Maehara, H. Baba, T. Okamura, and K. Sugimachi

The Second Department of Surgery, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

Received 14 March 1991/Accepted 9 August 1991

Summary. Relationships between in vitro chemosensitivity and cell nuclear DNA content were investigated in malignant cells from 41 patients exhibiting advanced gastric carcinoma. The chemosensitivity was evaluated by measuring the succinate dehydrogenase (SD) activity in drug-exposed cancer cells and the DNA content was microspectrophotometrically determined. Following exposure of malignant tissue to carboquone (CQ) and cisplatin (DDP), the mean SD activity in cells displaying a relatively regular DNA distribution (type II) was significantly higher than that in those exhibiting a widely scattered DNA distribution (type IV; $P < 0.01$ in CQ, $P < 0.05$ in DDP). A similar tendency was recognized in cells that were treated with aclacinomycin A (ACR), Adriamycin (ADM), and mitomycin C (MMC). Such a decrease in SD activity in cells exhibiting a type IV pattern was remarkable, especially in cases undifferentiated adenocarcinoma. Mitotic counting analysis revealed a significantly higher value for DNA pattern type IV as compared with the findings for type II ($P < 0.01$). These results demonstrate that gastric carcinoma displaying a high malignant potentiality shows a better response to antitumor drugs. Adjuvant chemotherapy prescribed following drug-sensitivity testing should be effective against such tumors.

Introduction

Cytophotometric DNA analysis provides a reliable indication of the prognosis for patients suffering from gastric carcinoma [10, 16, 17]. Tumors exhibiting a narrow DNA distribution are associated with a low malignant potential, whereas those displaying widely scattered DNA dispersion are linked to a high level of malignancy. The prognosis for lesions showing a high ploidy is poor, even in early-stage cancer. Therefore, effective adjuvant chemotherapy is necessary for the prolongation of patient survival.

An analysis of the chemosensitivity of gastric cancer tissues to various antitumor drugs has revealed significant differences among various tissues [4]. We therefore decided to determine whether gastric tissues exhibiting a high-ploidy DNA pattern would show a good response to antitumor drugs. The present study was warranted since many data on DNA in relation to prognosis or pathology are available, whereas reports of correlations between DNA distribution patterns and chemosensitivity are few. We examined interrelationships between DNA distribution patterns and chemosensitivity in tissue from patients exhibiting advanced gastric carcinoma.

Materials and methods

The present study involved tissues obtained from 41 patients exhibiting advanced gastric carcinoma (Table 1) who had been surgically treated at the Second Department of Surgery, Faculty of Medicine, Kyushu University, between 1984 and 1986. Neither irradiation nor chemotherapy had been prescribed for these patients.

The chemosensitivity of the gastric carcinoma tissues was determined using the succinate dehydrogenase inhibition (SDI) test [1, 19]. Tumor tissues were minced with scissors, passed through a number 32 stainless stell mesh into McCoy's 5A solution containing antibiotics, and then washed three times with this solution. The fragments were suspended in minimal essential medium supplemented with L-glutamine (292 mg/ml), 10% fetal calf serum, and antibiotics, were plated in 35-mm plastic dishes (3 dishes per test group and 4–6 per control), and were then incubated during exposure to antitumor drugs at 37° C in a humidified atmosphere containing 5% CO_2 for 3 days.

These fragments were then assayed for succinate dehydrogenase (SD) activity. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl 2H tetrazolium bromide (MTT) [24] was used as a hydrogen acceptor for the SD activity. The formazan formed from MTT was extracted with acetone containing 0.5% trichloroacetic acid and the absorbance of formazan was measured at 565 nm. The SD activity was expressed as the optical density (OD) per milligram of tissue protein. Tumor fragments displaying an OD value of 0.5 on day 0 were plated in separate dishes. The chemosensitivity was estimated by the percentage of SD activity in relation to that found in control cells, and the sensitivity was considered to be positive when the

Offprint requests to: A. Saito

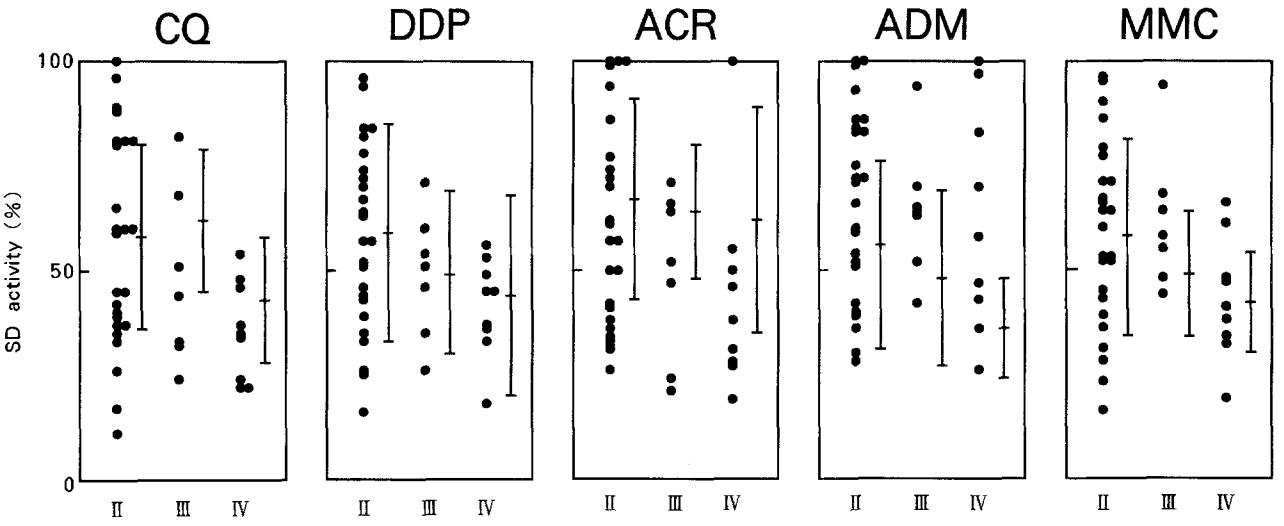

Fig. 1. SD activity and DNA distribution pattern (n = 41 cases). The chemosensitivity was indicated by the percentage of SD activity in drug-treated cells as compared with that in control cells. Points represent mean values ± standard deviation. *II, III, and IV* represent DNA distribution patterns. Significant differences were noted between pattern types II and IV following exposure to CQ (P <0.01) and DDP (P <0.05)

Table 1. Characteristics of patients exhibiting gastric carcinoma

Characteristic	Patients (n)
Sex:	
M	23 (56.1%)
F	18 (43.9%)
Age (years):	
Range	20–84
Mean	59.8
Tumor size (cm):	
Range	2–18.8
Mean	9.6
Location:	
Fundic	14 (41.2%)
Intermediate	10 (29.4%)
Pyloric	10 (29.4%)
Lymph node metastasis:	
Positive	29 (85.3%)
Negative	5 (14.7%)

SD activity in the drug-treated cells decreased to <50% of the control value [2, 22].

The antitumor drugs used included carboquone (CQ, 1 μg/ml), Adriamycin (ADM, 4 μg/ml), mitomycin C (MMC, 10 μg/ml), aclacinomycin A (ACR, 4 μg/ml), and cisplatin (DDP, 20 μg/ml, which were tested at 10 times the peak plasma concentration [16, 19]. The sources of these drugs were as follows: CQ, Sankyo Co., Ltd., Tokyo; ACR, Sanraku-Ocean Co., Ltd., Tokyo; ADM and MMC, Kyowa Hakko Co., Ltd., Tokyo; DDP, Nihon Kayaku Co., Ltd., Tokyo.

For measurement of the cell nuclear DNA content in the resected specimens, 10-μm-thick paraffin sections were prepared from the portion just adjacent to the hematoxylin eosin-stained section and Feulgen staining was then done using Naora's method [25]. Cell nuclear DNA content was measured by a microspectrophotometer (MPV3, Leitz, FRG) using the two-wavelength method [23]. Data processing was done on a personal computer (HP-85, Hewlett-Packard Co., Corvallis, Ore.) combined with a microspectrophotometer. A mean of 25 stromal lymphocytes was used as the control value for the normal diploid content (2c) in determinations of variation in the DNA content of cancer cells.

Based on measurements of the DNA content of 100 cancer cells in each lesion, the DNA distribution patterns were classified into 4 types [17] according to the degree of dispersion on the DNA histogram as follows:

1. Type I – most of the cells (>90%) exhibited DNA content values under 4c, with or without the scattering of cells being confined to 6c, and there were no cells showing values of over 6c.
2. Type II – although the proportion of cells exhibiting a value over 4c surpassed 10%, those displaying a value over 6c did not exceed 10%.
3. Type III – the proportion of cells showing a value over 6c surpassed 10% but did not surpass 20%.
4. Type IV – The cells showed a broadly scattered DNA histogram, and the proportion of cells exhibiting a value over 6c surpassed 20%.

Histological types were classified either as undifferentiated carcinoma or as differentiated carcinoma according to the criteria proposed by Sugano et al. [31]. The mitotic index (MI) was determined as follows. The number of tumor cells and the number of mitoses were counted in 10 high-power fields (HPF) at 400/slide. The MI was calculated as

$$\frac{\text{Mitoses/10 HPF}}{\text{Total number of tumor cells/10 HPF}} \times 1000 \text{ (number of mitoses/1000 cells)}$$

Student's t-test was used for statistical evaluations.

Results

Tissues from 25 patients exhibited a type II DNA distribution pattern, those from 7 cases displayed a type III pattern, and those from the remaining 9 cases showed a type IV pattern. The relationship between SD activity and DNA distribution patterns is summarized in Fig. 1. Following treatment with CQ, the mean SD value was 56.3 for cells exhibiting type II patterns, which was significantly higher than the 35.6 found for those displaying type IV distribution (P <0.01). After incubation with DDP, the decrease in SD activity was significant in cells showing type IV patterns as compared with those exhibiting type II distribution (P <0.05). A similar tendency was found for the other drugs, although the differences were not statistically significant. These cases were further studied from the viewpoint of histological types. In the differentiated-type cells, we found no difference in the SD activities among cells

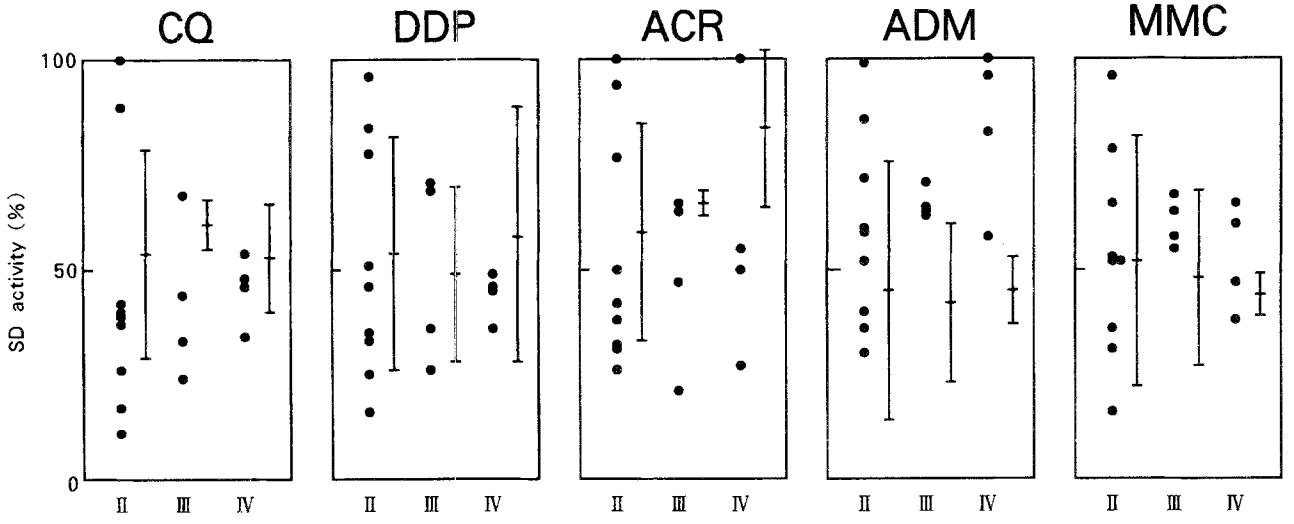

Fig. 2. SD activity and DNA distribution pattern (differentiated type, 17 cases). For definition of symbols, see Fig. 1

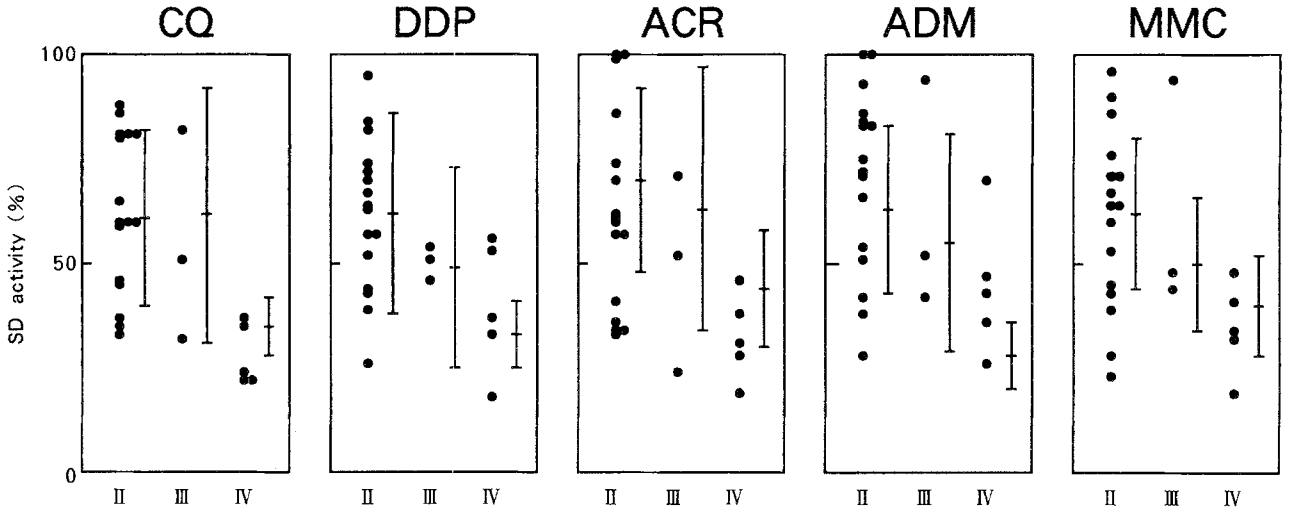

Fig. 3. SD activity and DNA distribution pattern (undifferentiated type, 24 cases). For definition of symbols, see Fig. 1. Significant differences were noted between pattern types II and IV following exposure to CQ (P <0.01) and the other four drugs (P <0.05)

Table 2. DNA distribution patterns and MI

DNA patterns	Cases (n)	Mean MI
All cases:		
Type II	25	5 ±3.53
Type III	7	10.3 ±4.95**
Type IV	9	10.48±5.29**
Differentiated type:		
Type II	9	7.12±3.35
Type III	4	9.76±4.75
Type IV	4	12.12±7.56
Undifferentiated type:		
Type II	16	3.81±3.12
Type III	3	11.02±6.2**
Type IV	5	9.17±2.86**

* Statistically significant differences as compared with type II (P <0.01, Student's t-test)

displaying different DNA distribution patterns (Fig. 2). However, in the undifferentiated-type cells, there were statistically significant differences in the SD activity between cells showing types II patterns and those exhibiting type IV distribution, with a significance value of P <0.01 being found for those exposed to CQ and that of P <0.05 being determined for those exposed to the other four drugs (Fig. 3).

Next, we examined the MI to investigate the cell kinetics. The average MI value increased in parallel with increasing degrees of DNA distribution from type II to type IV. The mean MI value was 5 in cells exhibiting type II patterns, 10.3 in those displaying type III distribution, and 10.48 in those showing type IV patterns. Statistically significant differences were observed in the findings for distribution types III vs II and types IV vs II (P <0.01). Table 2 summarizes these results. In the differentiated-type cells, there were no significant differences among individual DNA patterns, whereas the undifferentiated-type cells revealed remarkable differences in mean MI

Table 3. Chemosensitivity and MI

Chemosensitivity	MI				
	CQ	DDP	ACR	ADM	MMC
Positive	8.7 ±5.44*	7.97±5.55	9.19±5.56*	7.51±5.32	7.96±5.71
Negative	4.86±2.82*	6.11±4.10	4.92±2.8*	5.86±3.55	6.5 ±4.23

* Statistically significant differences in MI between positive and negative chemosensitivity (P <0.01, Student's t-test)
CQ, Carboquone; DDP, cisplatin; ACR, aclacinomycin A; ADM, Adriamycin; MMC, mitomycin C

values. The mean MI value for pattern type II was 3.81, which was significantly lower than those found for distribution types III and IV (P <0.01).

The relationship between the SD activity and the MI is summarized in Table 3. The MI was higher in chemosensitivity-positive tissues than in chemosensitivity-negative tissues following exposure to ACR and CQ (P <0.01). A similar tendency was observed following exposure to the other three drugs.

Discussion

Although radical surgery such as extended resection or prophylactic lymph node dissection has led to a considerable improvement in the resectability of gastric carcinoma, there are limitations in the case of advanced disease [3, 7, 13, 18, 29]. For such patients, the 5-year survival ranges from 23% to 40% [3, 5, 9, 13, 28]. According to studies on the prognosis of gastric cancer patients according to DNA analysis, individuals exhibiting hyperploid tumors (high ploidy) showed significant rates of recurrence as compared with those displaying near diploid tumors (low ploidy) [10, 16, 17]. Therefore, intensive adjuvant chemotherapy must be designed for patients presenting with advanced carcinoma, especially those exhibiting highly malignant disease characterized by widely scattered DNA values.

In Japan, various antitumor drugs have been prescribed for postoperative adjuvant chemotherapy. Chemosensitivity tests can serve as an indicator of the efficacy of antitumor drugs for the prolongation of survival [6, 8, 11, 26, 32]. There is general agreement that SD is an adenosine triphosphate (ATP)-producing key enzyme of the citric cycle and that the activity of this enzyme correlates well with cell viability [14, 15] as determined by the ATP assay [2, 21] and the dye exclusion method [21]. In esophageal carcinoma, SD activity correlates well with the clinical effectiveness of hyperthermochemoradiotherapy [27]. The SDI test is a simple, economical, and rapid technique for screening of antitumor drugs [20]. In the current series, we used this method to evaluate drug sensitivity and noted a correlation between the DNA profile and the SD activity in cases of advanced gastric carcinoma.

There was a marked decrease in SD activity in the type IV tumors, especially in those consisting of undifferentiated tissues. These findings are compatible with the results of Maehara et al. [20], who found that undifferentiated tissues of gastric cancer were more sensitive to antitumor drugs than were well-differentiated tissues. Subsequent analysis of the MI, an approach that has long been used by pathologists as an indicator of proliferative activity [30], revealed that the highly malignant type of carcinoma exhibiting widely scattered DNA values was characterized by high mitotic activity as compared with findings in tissues displaying a narrow distribution of DNA. Kim and Kim [12] reported that tumors containing a high proportion of S-phase fraction showed a better response to antitumor drugs. Considering these observations, the high drug sensitivity of type IV tumors may be due to enhanced DNA synthesis and replication as reflected by their mitotic activity.

A carcinoma exhibiting a high ploidy shows a strong malignant potential for lymph node metastasis, hematogenous metastasis, and invasion into surrounding tissues and hence, a low incidence of resectability [10, 16, 17]. As gastric tissues displaying widely scattered DNA values were more responsive to the drugs, in vivo clinical trials of specific adjuvant chemotherapy based on drug sensitivity should be considered.

References

1. Anai H, Maehara Y, Kusumoto H, Sugimachi K (1987) Comparison between succinate dehydrogenase inhibition test and subrenal capsule assay for chemosensitivity testing. Oncology 44: 115
2. Anai H, Maehara Y, Kusumoto H, Sugimachi K (1987) Comparison of the chemosensitivity of human neoplastic tissues between succinate dehydrogenase inhibition test and ATP assay. Clin Chim Acta 166: 107
3. Baba H, Korenaga D, Okamura T, Saito A, Sugimachi K (1989) Prognostic factors in gastric cancer with serosal invasion. Univariate and multivariate analyses. Arch Surg 124: 1061
4. Bertelsen CA, Sondak VK, Mann BD, Korn EL, Kern DH (1984) Chemosensitivity testing of human solid tumors – a review of 1582 assays with 258 clinical correlations. Cancer 53: 1240
5. Bizer LS (1983) Adenocarcinoma of the stomach: current results of treatment. Cancer 51: 743
6. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47: 936
7. Dent DM, Madden MV, Price SK (1988) Randomized comparison of R_1 and R_2 gastrectomy for gastric carcinoma. Br J Surg 75: 110
8. Griffin TW, Gogden AE, Reich SD (1985) Initial clinical trial of the subrenal capsule assay as a predictor of tumor response to chemotherapy. Cancer 52: 615
9. Haraguchi M, Okamura T, Sugimachi K (1987) Accurate prognostic value of morphovolumetric analysis of advanced carcinoma of the stomach. Surg Gynecol Obstet 164: 335
10. Inokuchi K, Kodama Y, Sasaki O, Kamegawa T (1983) Differentiation of growth patterns of early gastric carcinoma determined by cytophotometric DNA analysis. Cancer 51: 1138

11. Jones CA, Tsukamoto T, O'Brien PC (1985) Soft agarose culture human tumor colony forming assay for drug sensitivity testin: [^{3}H]-thymidine incorporation vs colony counting. Br J Cancer 52: 303
12. Kim SH, Kim JH (1972) Lethal effect of Adriamycin on the division cycle of HeLa cells. Cancer Res 43: 323
13. Kodama Y, Sugimachi K, Soejima K, Matsusaka T, Inokuchi K (1981) Evaluation of extensive lymph node dissection for carcinoma of the stomach. World J Surg 5: 241
14. Kondo T (1971) Prediction of response of tumor and host to cancer chemotherapy. Natl Cancer Inst Monogr 34: 251
15. Kondo T, Imamura T, Ichihashi H (1986) In vitro test sensitivity of tumor carcinostatic agents. Gann 57: 113
16. Korenaga D, Mori M, Okamura T, Sugimachi K, Enjoji M (1986) DNA ploidy in clinical malignant gastric lesions less then 5 mm in diameter. Cancer 58: 2542
17. Korenaga D, Okamura T, Saito A, Baba H, Sugimachi K (1988) The DNA ploidy is closely linked to tumor invasion, lymph node metastasis and prognosis in clinical gastric cancer. Cancer 62: 309
18. Korenaga D, Okamura T, Baba H, Saito A, Sugimachi K (1988) Results of resection of gastric cancer extending to adjacent organs. Br J Surg 75: 12
19. Maehara Y, Anai H, Tamada R, Sugimachi K (1986) Partial hepatectomy alters sensitivity of rat hepatocytes to 5-fluorouracil. Cancer Lett 31: 227
20. Maehara Y, Anai H, Kusumoto H, Sugimachi K (1987) Poorly differentiated human gastric carcinoma is more sensitive to antitumor drugs than is well differentiated carcinoma. Eur J Surg Oncol 13: 203
21. Maehara Y, Anai H, Tamada R, Sugimachi K (1987) The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. Eur J Cancer Clin Oncol 23: 273
22. Maehara Y, Emi Y, Sakaguchi Y, Kusumoto H, Kakeji Y, Kohnoe S, Sugimachi K (1990) Estrogen-receptor-negative breast cancer tissue is chemosensitive in vitro compared with estrogen-receptor-positive tissues. Eur Surg Res 22: 50
23. Mendersohn ML (1958) The two-wavelength method of microspectrophotometry: II. A set of tables to facilitate the calculations. J Cell Biol 4: 415
24. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival. Application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55
25. Naora H (1955) Microspectrophotometry of cell nucleus stained by Feulgen reaction: I. Microspectrophotometric apparatus without Schwarzschild-Villiger effect. Exp Cell Res 8: 259
26. Noso Y, Niimi K, Hirabayashi N (1987) Clinical studies on a new screening assay for anticancer agents using nude mice and isotopic evaluation. Cancer Res 47: 6418
27. Ohno S, Morita M, Tsutsui S, Matsuura H, Kuwano H, Maehara Y, Sugimachi K (1990) Correlation between hyperthermochemoradiosensitivity and clinical effect in carcinoma of the esophagus. Surg Gynecol Obstet 171: 472
28. Pagnini CA, Rugge M (1985) Advanced gastric cancer and prognosis. Virchows Arch [A] 406 (2): 213
29. Papachristou DN, Shiu MH (1981) Management by en bloc multiple organ resection of carcinoma of the stomach invading adjacent organs. Surg Gynecol Obstet 152: 483
30. Steel GG (1977) Growth kinetics of tumours: cell population kinetics in relation to the growth and treatment of cancer. Clarendon Press, Oxford
31. Sugano H, Nakamura K, Kato Y (1982) Pathological studies of human gastric cancer. Acta Pathol Jpn 32: 329
32. Weisenthal LM, Lippmann ME (1985) Clonogenic and nonclonogenic in vitro chemosensitivity assays. Cancer Treat Rep 69: 615