

Review

## Altered Deoxyribonuclease Activity in Cancer Cells and its Role in Non Toxic Adjuvant Cancer Therapy with Mixed Vitamins C and K<sub>3</sub>

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**Abstract.** *The alterations of deoxyribonuclease DNase activity in cancer cells were the basis of the utilization of mixed vitamins C and K<sub>3</sub> in a nontoxic, adjuvant cancer therapy. In order to localize exactly the altered activities of DNase in cancer cells, histochemical methods were utilized. The deficiency of alkaline and acid DNase activity appeared to be characteristic for non-necrotic cells of malignant human and animal tumors. This enzymatic deficiency appeared in experimental carcinogenesis before the phenotypic signs of malignancy. Tumor promoters directly reduced the activity of both DNases. The incidence of spontaneous malignant human and animal tumors appeared to be inversely proportional to the intensity of the activity of both DNases in normal cells and tissues from which these tumors were derived. The fact that alkaline and acid DNase activity was reactivated during the spontaneous and therapeutically induced necrosis of cancer cells suggests that this enzymatic deficiency of DNase activity in cancer cells was due to the action of specific inhibitors of DNases. Characteristic variations of serum alkaline DNase activity in positive responders to therapy, examined in more than 800 cancer-bearing patients, may be the basis for the development of a useful test for therapeutic prognosis and for monitoring of cancer bearing patients. Acid DNase was selectively reactivated in malignant tumor cells by vitamin C (sodium ascorbate), whereas alkaline DNase was reactivated by vitamin K<sub>3</sub>. Joint vitamin C and K<sub>3</sub> administration*

*produced in vitro and in vivo tumor growth inhibition, potentiation and sensitization of chemo- and/or radiotherapy and a decrease in the number of metastases in animals with experimental tumors. Joint vitamin C and K<sub>3</sub> administration may be considered as a possible new, non-toxic, adjuvant cancer therapy, which can be easily introduced into the classic protocols of clinical cancer therapy without any supplementary risk for patients.*

The main means for cancer therapy after surgical operation are chemo- or -radiotherapy. Factors which increase the effectiveness of cancer chemo- or radiotherapy are very useful cancer adjuvant therapy and are particularly important when non-toxic for cancer-bearing patients. Such value has been ascribed to the properties and action of the mixture of vitamins C and K<sub>3</sub>, which are proposed as a new, non-toxic, adjuvant cancer therapy. To understand the basis of our choice of this vitamin mixture as a means of adjuvant cancer therapy, a summary of our investigations on altered deoxyribonuclease (DNase) activity and its role in cancer is necessary.

### Assessment of DNase Activity

In order to understand the possible role of the activity of DNases, the examination of their detailed cellular localization was necessary. For this aim, the utilization of histochemical methods was necessary. To detect the activity of alkaline and acid DNase separately, histochemical methods based on Gomoris lead nitrate technique were utilized (1, 2). The activity of alkaline DNase is the result of a group of enzymes which depolymerise DNA in alkaline conditions, whereas that of acid DNase, a group of enzymes with their optimal activity in acid pH. The spectrophotometrical method for biochemical measurement of the serum alkaline DNase activity was utilized according to Loisel and Carrier (3). This method enabled the quantitative analysis of the variations of this enzyme activity in treated cancer-bearing patients.

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### Altered DNase Activity and its Role in Different Cancer Problems

Roger Daoust (4) was the first investigator to find that the activity of DNase was deficient in more than 60 malignant human and animal tumors. This discovery was confirmed using histochemical methods for the activity of alkaline and acid DNases in non-necrotic cells of malignant tumors of human digestive tract (5) and of human central nervous system (6). Similar deficiency of the activity of alkaline and acid DNase was found in malignant tumor cells in four types of experimental carcinogenesis in rats in: (i) kidneys (7), (ii) central nervous system (5), (iii) in stomach (8), (iv) in liver (9-12).

Such DNase deficiency appeared at very early stages of carcinogenesis, before the appearance of malignant cells, proving that it plays an important role in the mechanisms of malignant transformation (13) and is not a secondary marker of it. This DNase deficiency was also induced by tumor promoters in mouse skin (14) and in rat liver (15-17).

The alkaline and acid DNase activity was histochemically examined in the cells of rat and human central nervous system and in digestive tube and it was compared to the spontaneous incidence of tumors originating from these cells. It was found that the activity of DNases was very weak in glial cells from which most brain tumors originate, while the neurons demonstrated very intense activity of DNases and almost complete resistance to malignant transformation (18). Similarly, in rat and human digestive tube, the lowest activity of DNases was detected in the epithelial cells of the large intestine mucosa, the most frequent site of digestive tube carcinomas (64%), whereas the highest activity of alkaline and acid DNases was found in the epithelial cells of the mucosa of the small intestine, where human digestive tube carcinomas are very rare (0.9%), (19, 20).

These observations permitted the proposal of a hypothesis that the spontaneous incidence of malignant tumors is inversely proportional to the activity of alkaline and acid DNases in the normal cells from which these tumors originated.

### Reactivation of Alkaline and Acid DNase in Malignant Tumors

The histochemical pattern of alkaline and acid DNase activity in the foci of spontaneous necrosis of malignant tumors indicated a reactivation of these enzymes at early stages of necrosis. A distinct reactivation of DNase could be seen on the periphery of necrotic foci, whereas in the central area of such foci the activity of these enzymes was lacking most probably due to their inactivation by the autolytic process (5, 6, 21).

These variations mentioned in DNase activities were confirmed in the excised specimens of tumors following the *in vitro* induction of tumor cell necrosis or after the *in vivo*

performed tumor irradiation or treatment with cytotoxic drugs. Under these conditions, the reactivation of alkaline DNase appeared sooner and was of shorter duration than that of acid DNase (21, 22). These observations indicated that different mechanisms were involved in the deficiency of alkaline and acid DNase in malignant tumors and that this deficiency in tumors is a reversible phenomenon, most probably produced by natural inhibitors of these enzymes. The existence of such natural inhibitors in DNase-deficient tumors was demonstrated in a histochemical investigation, in which the usually very active alkaline and acid DNases in normal rat liver were completely inhibited after *in vitro* incubation of normal rat liver slices in a homogenate from rat hepatocarcinoma (21). Similar results were obtained using biochemical techniques by Loiselle and Carrier (3).

In experiments of two transplantable rat tumors, an intense reactivation of both DNases was observed following cyclophosphamide injection only in the tumor sensitive to cyclophosphamide treatment ISIS<sub>130</sub> (immunoglobulin-secreting immunocytoma<sub>130</sub>). DNase reactivation did not occur in the tumor which was resistant to cyclophosphamide treatment (ISIS<sub>208</sub>) (22).

These histochemical observations suggested the possibility of developing a biochemical test for therapeutic prognosis and clinical monitoring of cancer patients, based on characteristic variations of serum alkaline DNase activity (SADA). Positive results concerning such a test were obtained in more than 800 cancer-bearing patients examined in several university clinics in Belgium, France and Sweden (23-32).

Such characteristic variations of SADA were correlated with the response of the tumor to treatment. In positive responders to treatment, SADA decreased during the first days following therapy (phase I) and then increased during the weeks after treatment to a level equal to or higher than that before treatment (phase II). The maintenance of this high level of SADA for months after treatment accompanied the remission of the cancer process (phase III). A sudden decrease of SADA during the remission period preceded the recurrence of cancer by days or weeks. Negative responders to cancer treatment did not demonstrate such specific SADA variations.

### Involvement of DNase Reactivation in Tumor Treatment

Since DNase reactivation appeared to be linked to spontaneous or induced tumor necrosis and regression (21, 22), compounds which reactivate DNases in tumor cells should offer some potential for therapeutic intervention in cancer.

Indeed, it has been found that vitamin K<sub>3</sub> (2-methyl-1,4-naphthoquinone) selectively reactivated alkaline DNase in malignant tumors, whereas vitamin C (ascorbic acid or

Table I. Therapeutic effect of vitamin C-vitamin K<sub>3</sub> associated with a single intraperitoneal dose of different cytotoxic drugs administered to ascitic TLT-bearing mice.

Treatment	Parameters of therapeutic efficacy			
	Day of the first death	MST	T/C	ILS (%)
None	13	18.2		
CK3 alone	10	23.1	1.458	+45.8
Cyclophosphamide alone	19	24.7	1.127	+12.8
Cyclophosphamide → CK3	23	30.0	1.369	+36.9
CK3 → Cyclophosphamide	25	32.7	1.493	+49.3
Catalase → CK3 → Cyclophosphamide	3	24.0	1.200	+20.0
Procarbazine alone	18	22.4	1.260	+26.5
CK3 → Procarbazine	18	31.5	1.770	+77.9
Asparaginase alone	15	19.5	1.100	+10.2
CK3 → Asparaginase	15	30.4	1.700	+71.7
Vinblastine alone	19	20.9	1.870	+16.1
Vinblastine → CK3	11	22.5	1.250	+25.0
CK3 → Vinblastine	20	33.8	1.870	+87.0
Adriamycin alone	9	21.1	1.180	+18.5
Adriamycin → CK3	13	30.3	1.700	+70.2
CK3 → Adriamycin	27	33.6	1.880	+88.8
5-Fluorouracil	17	22.0	1.157	+15.7
5-Fluorouracil → CK3	18	32.9	1.750	+73.1
CK3 → 5-Fluorouracil	33	46.2	2.430	+143.0

MST, Mean survival time (days); T/C, MST treated group/MST control group; ILS, percentage increase in lifespan.

sodium ascorbate) exclusively reactivated acid DNase (21). Administration of a mixture of both vitamins as a single intraperitoneal dose of 1 g/kg body weight of vitamin C and 0.01 g/kg body weight of vitamin K<sub>3</sub> considerably inhibited the ascitic tumor growth in mice (33). This therapeutic effect, calculated in percentage increase of lifespan (ILS), had a synergistic character (45%) when compared to the separate effect of vitamin C (14.7%) and K<sub>3</sub> (1.07%).

This vitamin combination has also been evaluated as a possible adjuvant treatment in chemo- and/or radiotherapy of cancer. Experiments were performed on the ascitic form of a transplantable liver tumor (TLT) (34) in young, adult male mice of NMRI strain (33). Forty-eight hours after intraperitoneal transplantation of 10<sup>6</sup> TLT cells, a single subtherapeutic dose of one of the 6 drugs commonly utilized in human cancer therapy was intraperitoneally injected. Vitamins C and K<sub>3</sub> water solutions at a dose of 1g/kg for vitamin C and 10 mg/kg for vitamin K<sub>3</sub> were intraperitoneally injected 24 and 3 hours before or after the single dose of anticancerous chemotherapeutic drugs. Mortality rates, the mean survival time (MST), the MST in the treated group divided by that in controls (T/C), and the ILS for each group of 10 to 12 ascitic tumor-bearing mice were calculated according to the instructions of the National Cancer Institute (NCI) of U.S.A. Intraperitoneal administration of combined vitamin C and K<sub>3</sub> directly before a single intraperitoneal,

subtherapeutic dose of different cytotoxic drugs utilized in human cancer therapy produced a distinct (in many cases synergistic) potentiation of the therapeutic effect in the ascitic form of transplantable mouse liver tumor bearing-mice (Table I) (33). Amongst these investigated anticancer drugs were: cyclophosphamide (Endoxan; Asta, Bielefeld, Germany); vinblastine (Velbe; Eli-Lilly, Indianapolis, USA); adriamycin (Adriablastina; Farmitalia-Carlo Erba, Milano, Italy); procarbazine hydrochloride (Natulan; Roche, Basel, Switzerland); asparaginase (Crasnitine; Bayer, Leverkusen, Germany); 5-fluorouracil (Roche, Basel, Switzerland). This adjuvant treatment did not increase the systemic, or the organ toxicity which usually accompanies these cytotoxic drugs (33). The same adjuvant treatment may also sensitize tumors which are resistant to some cytotoxic drugs, as in the case of vincristine sulphate (oncovin; Eli-Lilly, Indianapolis, U.S.A.) to which the investigated tumor was resistant (35).

*In vitro* experiments with three human tumor cell lines (MCF-7 breast carcinoma, K.B. oral epidermoid carcinoma and AN3-CA endometrial adenocarcinoma) confirmed the synergistic potentiation of chemotherapy and inhibition of tumor growth induced by joint vitamin C and K<sub>3</sub> administration (36, 37). Joint vitamin C and K<sub>3</sub> administration also potentiated the effects of radiotherapy induced by 20 to 40 Gy of X-rays in solid intramuscularly transplanted mouse liver tumor (TLT) (38).

Dietary treatment with combined vitamins C and K<sub>3</sub> considerably inhibited the development of metastases in lungs and lymphatic nodules of TLT. tumor intramuscularly implanted in mice (39).

Amongst the different mechanisms which may be involved in this therapeutic effect of joint vitamin C and K<sub>3</sub> against cancer, the most plausible is a stimulation of the redox cycling system which produces hydrogen peroxide and other active oxygen species which are involved in cell membrane lipid peroxidation, DNase activation and DNA breaks, leading to cell death (40-44).

Moreover, the most important fact is that this action of joint vitamin C and K<sub>3</sub> administration appeared to be selective for cancer cells. As opposed to normal organs and tissues, cancer cells are usually deficient in catalase, superoxide dismutase and/or glutathione peroxidase, which constitute the cellular defense system against free radicals (45, 46). This hypothesis is supported by the fact that simultaneous administration of vitamin C and K<sub>3</sub> with catalase suppressed the therapy potentiating and sensitizing effect of these vitamins. Moreover, no additional systemic and organ toxicity was induced by joint vitamin C and K<sub>3</sub> administration (33, 35, 37). Vitamin C is selectively absorbed and concentrated in cancer cells, providing the basis for its being utilized in cancer therapy (46), especially when administered intravenously (47). These facts may encourage the utilization of CK<sub>3</sub> vitamins in cancer therapy, mainly when accompanied by cancer chemo- or radiotherapy, ie as an efficient cancer adjuvant therapy.

The tumor growth-inhibiting and tumor therapy-potentiating or -sensitizing action of joint vitamin C and K<sub>3</sub> administration produced tumor cell necrosis, apoptosis, or, as recently described by Gilloteaux *et al.* (40, 47, 48), a new kind of cell death called autoschizis. It should be added that numerous articles described the activation of endonucleases (DNases) as an early event leading to the cell death, mainly by apoptosis (49). It has been described that the chromatin of apoptotic cells was broken up by specific endonuclease (DNase) into nucleosomes (fragments of 190 base pairs of DNA) (50).

Recent publications described in more detail the possible mechanisms involved in anticancer activities of the mixed vitamins C and K<sub>3</sub> (51-59). American researchers from Anderson Cancer Center in Huston recently confirmed our results of cancer chemotherapy potentiation with mixed vitamins C and K<sub>3</sub> and suggested this new adjuvant cancer therapy be examined in clinics (60).

### General Conclusions and Perspectives

The investigations of the histochemical or biochemical activity of alkaline and acid DNase were the basis for the introduction of vitamins C and K<sub>3</sub> into adjuvant cancer therapy and led to the following conclusions.

Alkaline and acid DNase activity was inhibited in the non-necrotic cells of malignant tumors. This inhibition considerably preceded the appearance of the morphological signs of malignancy in experimental carcinogenesis and was induced by tumor promoters. The finding of reduced DNase activity in different tissues and cells may help to detect potential promoters or a tumor-promoting state. Such investigation may indicate new ways for cancer prevention. Moreover, the incidence of spontaneous malignant tumors in humans and in experimental animals appeared to be inversely proportional to the activity of alkaline and acid DNase in normal tissues and cells from which these tumors originated. Thus, it may be suggested that normal cells having lower DNase activity are more predisposed to malignant transformation due to their less active DNA repair mechanism. The fact that the typical deficiency of alkaline and acid DNase activity in malignant tumor cells is reactivated in both spontaneous and therapeutically induced necrosis of tumor cells suggests that this enzymatic deficiency was due to the action of some specific inhibitors of DNases.

Variations of serum alkaline DNase activity in cancer-bearing patients demonstrated characteristic curves only in positive responders to chemo- or radiotherapy. These variations may be considered as a potential prognostic test for positive tumor treatment and as a sensitive marker for monitoring cancer bearing patients. Its use in clinics may be very helpful.

Joint administration of vitamin C and K<sub>3</sub>, which reactivated acid and alkaline DNase inhibited in cancer cells, caused tumor growth inhibition, a decrease of metastasis and potentiated or sensitized the chemotherapy both *in vitro* and *in vivo*. This joint vitamin administration also potentiated the effects of radiotherapy of malignant tumors. Therefore, a combined vitamin C and K<sub>3</sub> administration may be considered as a new non-toxic, adjuvant cancer therapy, which can be easily introduced into the classic protocols of clinical cancer therapy without any supplementary risk for patients.

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### References

- 1 Taper HS: La détection histochimique de la désoxyribonucléase alcaline. *Ann Histochem* 13: 301-317, 1968.
- 2 Vorbrodt A: Histochemical studies on the intracellular localization of acid deoxyribonucléase. *J Histochem Cytochem* 9: 647-655, 1961.
- 3 Loisel JM and Carrier R: Désoxyribonucléase sérique chez les cancéreux. *Rev Canad Biol* 22: 41-46, 1963.

- 4 Daoust R and Amano H: Ribonuclease and deoxyribonuclease activities in experimental and human tumors by histochemical substrate film method. *Cancer Res* 23: 131-134, 1963.
- 5 Taper HS: The relation between the histochemical activity of nucleases and neoplasms in rat and man. Vander, Louvain, 1975.
- 6 Taper HS, Brucher JM and Fort L: Alkaline and acid nuclease activity in the tumors of the human central nervous system. (Histochemical study). *Cancer* 28: 482-490, 1971.
- 7 Taper HS: L'activité des nucléases et phosphatases acides et alcalines dans les tumeurs rénales induites chez le rat par la diméthylnitrosamine (Etude histochimique). *Path Eur* 2: 406-420, 1967.
- 8 Fort L, Taper HS and Brucher JM: Gastric carcinogenesis induced in rats by methylnitrosourea (MNU). Morphology and histochemistry of nucleases. *Z Krebsforsch* 81: 51-62, 1974.
- 9 Taper HS, Fort L and Brucher JM: Histochemical activity of alkaline and acid nucleases in rat liver parenchyma during *N*-nitrosomorpholine carcinogenesis. *Cancer Res* 31: 913-916, 1971.
- 10 Taper HS and Bannasch P: Histochemical correlation between glycogen, nucleic acids and nucleases in preneoplastic lesions of rat liver after short-term administration of *N*-nitrosomorpholine *Z Krebsforsch* 87: 53-65, 1976.
- 11 Taper HS and Bannasch P: Histochemical correlation between so-called megalocytosis and preneoplastic lesions of rat liver after sublethal dose of *N*-nitrosomorpholine. *Eur J Cancer* 15: 189-196, 1979.
- 12 Lans M, de Gerlache J, Taper H, Pr at V and Roberfroid M: Phenobarbital as a promotor in the initiation/selection process of experimental rat hepatocarcinogenesis. *Carcinogenesis* 4: 141-144, 1983.
- 13 Cleaver JE, Bootsma D and Friedberg E: Human diseases with genetically altered DNA repair processes. *Genetics* 79: 215-225, 1975.
- 14 Taper HS: Induction of deficient acid DNase activity in mouse interfollicular epidermis by croton oil as a possible tumor-promoting mechanism. *Z Krebsforsch* 90: 197-210, 1977.
- 15 Fort L, Taper HS and Brucher JM: Morphological alterations and focal deficiency of the histochemical activity of acid and alkaline nucleases in rat liver after chronic administration of phenobarbital. *Beitr Pathol* 161: 363-375, 1977.
- 16 Taper H S, Lans M, de Gerlache J, Fort L and Roberfroid M: Morphological alterations and DNase deficiency in phenobarbital promotion of *N*-nitrosomorpholine initiated rat hepatocarcinogenesis. *Carcinogenesis* 4: 231-234, 1983.
- 17 Taper H, de Gerlache J, Lans M, Preat V and Roberfroid M: Estrogens and primary liver tumors. *In: Hormones and Sexual Factors in Human Cancer*. Wolff JP and Scott JS (eds). London, Elsevier Science Publ., pp. 163-174, 1984.
- 18 Taper, HS, Brucher JM and Doyen G: Histochemical study on alkaline and acid nucleases activity in the human brain compared to the incidence of malignant tumors. *J Neurol Sci* 12: 69-82, 1971.
- 19 Fort L and Taper HS: L'activit  histochimique des nucl ases alcalines et acides compar e   l'incidence des carcinomes dans le syst me digestif du rat. *Path Eur* 4: 41-57, 1969.
- 20 Fort L and Taper HS: Nucleases activity in different segments of the human digestive tube compared to the incidence of carcinomas (histochemical study). *Histochemie* 20: 150-158, 1969.
- 21 Taper HS: Reversibility of acid and alkaline deoxyribonuclease deficiency in malignant tumor cells. *J Histochem Cytochem* 29: 1053-1060, 1980.
- 22 Taper HS, Deckers CO and Deckers-Passau LO: Increase in nuclease activity as a possible means for detecting tumor cell sensitivity to anticancer agents. *Cancer* 47: 523-529, 1981.
- 23 Avall-Lundqvist E, Economidou-Karaoglou A, Sjovall K, Lans M, Taper HS, Roberfroid M, and Enneroth P: Serum alkaline DNase activity in normal or non hospitalized individuals. *Clin Chim Acta* 185: 35-44, 1989.
- 24 Dewez B, Lans M, Allaey V, Karaoglou A, Taper HS and Roberfroid M: Serum alkaline deoxyribonuclease activity, a sensitive marker for the therapeutic monitoring of cancer patients: Methodological aspects. *Europ J Clin Chem Biochem* 31: 793-797, 1993.
- 25 Economidou-Karaoglou A, Opsomer M, Petit G, Lans M and Roberfroid M: Variations in serum alkaline DNase activity: A new means for therapeutic monitoring of malignant lymphomas. *Cancer* 61: 1838-1843, 1988.
- 26 Economidou-Karaoglou A, Opsomer M, Petit G, Lans M, Taper HS and Roberfroid M: Characteristic variations of serum alkaline DNase activity in relation to response to therapy and tumor prognosis in human lung cancer. *Eur J Cancer Clin Oncol* 24: 1337-1343, 1988.
- 27 Economidou-Karaoglou A, Lans M, Taper HS, Michaux JL, and Roberfroid M: Variations in serum alkaline DNase activity: A new means to access early detection of relapse in patients treated for acute nonlymphoblastic leukaemia. *Blood* 74: 2730-273, 1989.
- 28 Economidou-Karaoglou A, Brasseur F, Lans M, Taper HS and Roberfroid M: Variations in serum alkaline DNase activity in rats during growth and treatment of tumors sensitive or resistant to therapy. *Int J Cancer* 43: 956-959, 1989.
- 29 Economidou-Karaoglou A, Opsomer M, Lans M, Taper HS, Deckers C and Roberfroid M: Predictive value of serum alkaline DNase activity variations in treatment of head and neck cancer. *Acta Oncol* 29: 163-166, 1990.
- 30 Rouillet B, Labadie M, Taper HS, Lans M, Economidou-Karaoglou A, Dewez B, Roberfroid M, Berdah JF, Bachellerie-Rhein B, Clavere P *et al*: Valeur predictive des variations de l'activit  s rique de la DNase alcaline dans le pronostic des cancers des voies a rodigestives sup rieures trait es par chimioth rapie. *Bull Cancer* 77: 675-680, 1990.
- 31 Taper HS, Lans M, Economidou A, de Gerlache J and Roberfroid M: A possible test of therapeutic prognosis of human tumors based on the detection of serum alkaline DNase variations. *Anticancer Res* 5: 600, 1985.
- 32 Taper HS, de Gerlache J, Lans M and Roberfroid M: Variations in serum DNase activity: A possible test for therapeutic prognosis of human tumors. *Anticancer Res* 6: 949-956, 1986.
- 33 Taper HS, de Gerlache J, Lans M and Roberfroid M: Non-toxic potentiation of cancer chemotherapy by combined C and K<sub>3</sub> vitamin pre-treatment. *Int J Cancer* 40: 575-579, 1987.
- 34 Cappucino JG, Brown GF, Mountain SM, Spencer S and Tarnowski GS: Chemotherapeutic studies on the new transplantable mouse liver tumor (Taper liver tumor). *Cancer Res* 26: 689-694, 1966.
- 35 Taper HS and Roberfroid M: Non toxic sensitization of cancer chemotherapy by combined vitamin C and K<sub>3</sub> pretreatment in a mouse tumor resistant to Oncovin. *Anticancer Res* 12: 1651-1654, 1992.

- 36 De Loecker W, Janssens J, Bonte J and Taper HS: Effects of sodium ascorbate (vitamin C) and 2-methyl-1,4-naphthoquinone (vitamin K<sub>3</sub>) treatment on human tumor cell growth *in vitro*. II. Synergism with combined chemotherapy action. *Anticancer Res* 13: 103-106, 1993.
- 37 Noto V, Taper HS, Yi-Hua J, Janssens H, Bonte J and De Loecker W: Effects of sodium ascorbate (vitamin C) and 2-methyl-1,4-naphthoquinone (vitamin K<sub>3</sub>) treatment on human tumor cell growth *in vitro*. *Cancer* 63: 901-906, 1989.
- 38 Taper HS, Keyeux A and Roberfroid M: Potentiation of radiotherapy by non-toxic pre-treatment with combined vitamin C and K<sub>3</sub> in mice bearing solid, transplantable tumor. *Anticancer Res* 16: 499-505, 1996.
- 39 Taper HS, Jamison J M, Gilloteaux J, Summers JL and Buc Calderon P: Inhibition of the development of metastases by dietary vitamin C/K<sub>3</sub> combination. *Life Sci* 75: 955-967, 2004.
- 40 Gilloteaux J, Jamison JM, Venugopal M, Giammar D and Summers JL: Scanning electron microscopy and transmission electron microscopy aspects of synergistic antitumor activity of vitamin C-vitamin K<sub>3</sub> combinations against human prostatic carcinoma cells. *Scanning Microscop Intern* 9: 159-173, 1995.
- 41 Jamison JM, Gilloteaux J, Venugopal, M Koch, JA, Sowick C, Shah R and Summers JL: Flow cytometric and ultrastructural aspects of the synergistic antitumor activity of vitamin C-vitamin K<sub>3</sub> combinations against prostatic carcinoma cells. *Tissue Cell* 28: 687-701, 1996.
- 42 Jamison JM, Gilloteaux J, Koch JA, Nicastro E, Doherty JJ, Jasso G, and Summers JL: Vitamin C and K<sub>3</sub>-induced oxidative stress in human prostate tumor cells: mitochondrial ultrastructural alterations. *Microscopy A. Microanalysis* 3(suppl 2): 23-24, 1997.
- 43 Venugopal M, Jamison J M, Gilloteaux J, Koch J A, Summers M, Hoke G, Sowick C and Summers JL: Synergistic antitumor activity of vitamin C and K<sub>3</sub> against human prostate carcinoma cell lines. *Cell Biol Int* 20: 787-797, 1996.
- 44 Venugopal M, Jamison JM, Gilloteaux J, Koch JA, Summers M, Giammar DM, Sowick C, and Summers J L: Synergistic antitumor activity of vitamins C and K<sub>3</sub> on human urologic tumor cell lines. *Life Sciences* 59: 1389-1400, 1996.
- 45 Sinha BK and Mimmaugh EG: Free radicals and anticancer drug resistance. Oxygen free radicals in the mechanisms of drug cytotoxicity and resistance by certain tumors. *Free Rad Biol Med* 8: 567-581, 1990.
- 46 Cameron E, Pauling L and Leibovitz B: Ascorbic acid and cancer: A review. *Cancer Res* 39: 663-681, 1979.
- 47 Padayatty SJ, Riordan HD, Hewitt SM, Katz A, Huffer LJ, Levine M: Intravenously administered vitamin C as cancer therapy: three cases. *Canad Med Assoc J* 174: 937-942, 2006.
- 48 Gilloteaux J, Jamison JM, Arnold D, Ervin E, Docherty JJ, Neal D and Summers JL: Cancer cell necrosis by autolysis: Synergism of antitumor activity of vitamin C: vitamin K<sub>3</sub> on human bladder carcinoma T24 cells. *Scanning* 20: 564-575, 1998.
- 49 Kauffman SJ: Apoptosis. Pharmacological implications and therapeutic opportunities. *In: Advances in Pharmacology* 41, San Diego, Academic Press, 1997.
- 50 Wyllie AH: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284: 555-556, 1980.
- 51 Taper HS, Jamison JM, Gilloteaux J, Gwin CA, Gordon T and Summers JL: *In vivo* reactivation of DNases in implanted human prostate tumors after administration of a vitamin C/K<sub>3</sub> combination. *J Histochem Cytochem* 49: 109-119, 2001.
- 52 Gilloteaux J, Jamison JM, Arnold D, Taper HS, von Grueningen UE and Summers JL: Microscopic aspects of autolysis cell death in human ovarian carcinoma (2774) cells following vitamin C, vitamin K<sub>3</sub> or vitamin C:K<sub>3</sub> treatment. *Microsc Microanal* 9: 311-329, 2003.
- 53 Buc Calderon P, Cadrobbi J, Marques C, Hong-Ngoc N, Jamison JM, Gilloteaux J, Summers JL and Taper HS: Potential therapeutic application of the association of vitamin C and K<sub>3</sub> in cancer treatment. *Cur Med Chem* 9: 2271-2285, 2002.
- 54 Verrax J, Cadrobbi J, Delvaux M, Jamison JL, Gilloteaux J, Summers JL, Taper HS and Buc Calderon P: The association of vitamins C and K<sub>3</sub> kills cancer cells mainly by autolysis, a novel form of cell death. Basis for their potential use as adjuvants in cancer therapy. *Eur J Med Chem* 38: 451-457, 2003.
- 55 Verrax J, Cadrobbi J, Marques C, Taper HS, Habraken Y, Piette J and Buc Calderon P: Ascorbate potentiates the cytotoxicity of menadione leading to an oxidative stress that kills cancer cells by a non-apoptotic caspase-3-independent form of cell death. *Apoptosis* 9: 225-235, 2004.
- 56 Verrax J, Bollen S, Delvaux M, Taper HS and Buc Calderon P: New insights about the potential application of the association of vitamins C (sodium ascorbate) and K<sub>3</sub> (menadione) as auxiliary therapy in cancer treatment. *Med Chem Rev* 2: 277-282, 2005.
- 57 Verrax J, Stockis J, Tison A, Taper HS and Buc Calderon P: Oxidative stress by ascorbate-menadione association kills K562 human chronic myelogenous leukaemia cells and inhibits its tumor growth in nude mice. *Biochem Pharmacol* 72: 671-680, 2006.
- 58 Verrax J, Vanbever S, Stockis J, Taper HS and Buc Calderon P: Role of glycolysis inhibition and poly (ADP-ribose) polymerase activation in necrotic-like cell death caused by ascorbate/menadione-induced oxidative stress in K562 human chronic myelogenous leukemic cells. *Int J Cancer* 120: 1192-1197, 2007.
- 59 Verrax J, Taper HS and Buc Calderon P: Targeting cancer cells by an oxidant-based therapy. *Curr Mol Pharmacol* 1: 80-92, 2008.
- 60 Kassouf W, Highshaw R, Nelkin G M, Dinney CP and Kamat AM: Vitamins C and K<sub>3</sub> sensitize human urothelial tumors to gemcitabine. *J Urol* 176: 1642-1647, 2006.

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