FROM THE NATIONAL INSTITUTE OF ONCOLOGY (DIRECTOR GENERAL: PROF. S. ECKHARDT), RÁTH GYÖRGY, H-1122 BUDAPEST, HUNGARY.

BASAL CELL CARCINOMA TREATED WITH MTDQ AND IRRADIATION

POLLÁK ZS., FODOR J., ERDÉLYI V., BIHARI Ö. and ECKHARDT S.

MTDQ (6,6′-methylene-bis-(2,2,4-trimethyl-1,2-dihydroquinoline)) seems to be a non-toxic radiation sensitizer antioxidant developed by BÄR et coll. (1971). The compound accumulates selectively in hypoxic tumor cells. Its biologic half life in human blood plasma is 48 to 56 hours when administered orally (BÄR et coll. 1971, 1975, 1977).

By means of simultaneous administration of MTDQ and radiation therapy, or premedication with MTDQ followed by combined treatment, tumor regression has been achieved in stage III and IV of neoplasms with relatively low radiation sensitivity such as malignant synovioma, fibrosarcoma, neurofibrosarcoma, epithelial malignant tumors of the head and neck, malignant melanoma, and carcinoma of the breast. The compound has proved suitable for repeated and prolonged administration. In patients with squamous cell carcinoma of the tongue and in Enzinger's sarcoma combined treatment has resulted in incomplete, though clinically appreciable and lasting regression (RODÉ et coll. 1976, 1977, ERDÉLYI et coll., to be published).

The aim of the present clinical trial was to determine whether MTDQ could reduce the radiation dose in the treatment of tumors with moderate sensitivity to radiation. Biochemical changes indicating the mode of action of the drug have also been examined.

Basal cell carcinoma of the skin was chosen for this clinical trial. This tumor has a relatively low sensitivity to radiation, but has histologic criteria of malignancy. It

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grows slowly and in general does not metastasize. Therefore, it might be an ideal tumor for testing of a radiation sensitizer. If a tumor remnant or recurrence should occur, due to the reduced dose, this growth can easily be removed surgically and does not involve any significant risk for the patient. Basal cell carcinoma of the skin can be cured by means of contact orthovoltage roentgen irradiation alone and the effect of the treatment can be established by simple inspection. Tumor tissue can also be easily excised, if required for biochemical analysis.

Lipid peroxidation of the organellum and cell membranes seems to be of importance in the process of carcinogenesis (MASON et coll. 1960). During the first part of carcinogenesis and during the period of metastases formation increased concentration of free radicals has been detected (EMANUEL 1973, SCHWARTZ et coll. 1973).

In the course of auto-oxidation of the polyunsaturated fatty acids and their esters malonaldehyde is formed both in vivo and in vitro (PRYOR 1975, PRYOR & STANLEY 1975). The malonaldehyde concentration may be used as a measure of the intensity of free radical reactions and is probably correlated to the phase of tumor progression. The presence of malonaldehyde has been demonstrated in transplanted tumors and during the course of chemical carcinogenesis (SHAMBERGER 1969, WATTENBERG 1973).

In preliminary trials increased malonaldehyde concentration has been found in untreated basal cell carcinoma of the skin, which has decreased after administration of MTDQ (FODOR et coll. 1977). In acidic medium malonaldehyde with thiobarbituric acid yields a characteristic color reaction with an absorption maximum at 532 nm. This color complex is a sensitive and specific marker of the unstable endoperoxides formed during auto-oxidation in biologic systems (GUTTERIDGE 1975, PRYOR).

In the course of the combined treatment with MTDQ and irradiation the concentration of MTDQ in the tumor tissue was determined, and also whether the malonaldehyde concentration in the tumor tissue changed during the treatment. A correlation between the concentration of the two substances should indicate a biologic effect of MTDQ.

Material and Methods

The material consisted of 65 adult patients. The diameter of the tumor did not exceed 20 mm and its thickness did not exceed 3 mm.

The patients were randomized into three groups and irradiated with 60 kV; filter 1 mm Al, FSD 15 cm, HVL 12 mm. Treatment regime 400 R/day (103 mC/kg), 5 treatments per week. Group I included 21 patients. Orthovoltage roentgen therapy was given with a conventional (curative) skin exposure amounting to 10×400 R (10×103 mC/kg). Group II (16 patients) was given 3×2 MTDQ tablets (1 320 mg) daily on 8 successive days before and during irradiation. The total skin exposure in these cases was 5×400 R (5×103 mC/kg). Group III (18 patients) received only 5×400 R (5×103 mC/kg).

Malonaldehyde and MTDQ concentrations in tumor tissue were determined in 2 patients with so-called twin basaliomas of the skin. In each of these patients one of

the tumors was removed before treatment for determination of the malonaldehyde concentration. MTDQ and malonaldehyde concentrations were determined in the other tumors on day 14 and day 30.

Malonaldehyde was determined as follows: The excised tumor tissue was weighed and rubbed with 1 to 2 g quartz sand and 4 ml phosphate buffer. After incubation for 1.5 h at 37°C 2 ml trichloracetic acid was added and the mixture centrifugalized (4 000 rpm). Two ml thiobarbituric acid solution was added to 3 ml pure solution and was kept for 10 min in a boiling water bath. After cooling, photometric analysis at 532 nm was performed. The results were obtained by means of a calibration curve recorded with standard malonaldehyde solutions.

MTDQ was determined as follows: The removed tumor tissue was weighed and rubbed with 1 to 2 g quartz sand and 5 to 10 ml benzene and kept for 16 h at room temperature in the dark, and shaken occasionally. Thereafter the mixture was filtered, washed with benzene and extracted twice with 5 ml 1 n hydrochloric acid. In the hydrochloric phase 5 ml benzene was added and the mixture alkalized with 1 n sodium hydroxide solution (pH 8–10). After shaking, the MTDQ-benzene mixture was separated and dried with sodium sulfate. To 2.5 ml benzene solution of MTDQ was added 0.5 ml ferric chloride reagent and photometric determination at 644 nm was performed 10 min later. The MTDQ concentration was determined by means of the calibration curve of standard MTDQ solutions.

Results

The results are summarized in Table 1. The effect of 2 000 R administered after MTDQ pretreatment was compared with the effect of the same dose without MTDQ premedication, as well as with the effect of irradiation with 4 000 R.

The results were evaluated by means of the χ^2 test. A significant difference was found between the therapeutic response obtained after MTDQ pretreatment and combined administration of MTDQ and irradiation with 2 000 R, and the response after the same radiation dose delivered without MTDQ ($p<0.05$). No significant difference was found between the therapeutic response obtained with 2 000 R + MTDQ treatment and that obtained with 4 000 R without MTDQ administration.

Table 1

Effect of MTDQ and irradiation

Treatment	No. of cases	Complete recovery	Residual tumor
10 × 400 R	21	15	6
5 × 400 R + MTDQ	26	21	5
5 × 400 R	18	9	9

Table 2

Malonaldehyde and MTDQ concentration (μg/g) in tumor tissue

	Before MTDQ	On day 14	On day 30
Malonaldehyde	1.5	1.17	0.5
MTDQ	—	11.5	14.0

The concentrations of MTDQ and malonaldehyde in the tumor tissue in the 2 cases with twin basaliomas appear in Table 2.

Discussion

Of the test methods suitable for assessing the effect of chemical radiation sensitizers the oxygen enhancement effect in bacterial cultures and in surviving cell cultures as well as in hypoxic human skin is in common use (ADAMS 1973). However, discrepant biologic properties and biochemical environment render extrapolation of results obtained in these experiments to human tumors difficult. Therefore, a search for well measurable parameters (markers) such as reduction of the effective radiation dose is required. Malonaldehyde represents not only a 'marker', it is also considered to be a carcinogenic substance (GUTTERIDGE). After the administration of different chemical substances, as well as in animals with transplanted tumors and in several human tumor tissues, for instance malignant melanoma, this highly reactive compound (ultimate carcinogen) can be detected (SHAMBERGER et coll. 1974).

Recent results indicate that in the course of auto-oxidation of lipids endoperoxide complexes and polymerisates are formed in vivo. Malonaldehyde yielding a characteristic color reaction with thiobarbituric acid is formed from these unstable metabolites in acidic medium (GUTTERIDGE).

The correlation between the decrease of the malonaldehyde concentration and the increase of MTDQ concentration in the tumor tissue confirms biochemically the action of MTDQ. The observation supports the hypothesis that the radical scavenger and peroxide-decomposing antioxidant reacts with the endoperoxides and forms inactive metabolites. On the other hand, in the course of these reactions oxygen is released in the tumor tissue (POLLÁK et coll. 1978). The correlation between the decrease of the malonaldehyde concentration and the duration of MTDQ medication confirms the previous experiences, i.e. an approximately constant plateau-like level of the effective serum concentration has been found between day 20 and day 40 of MTDQ medication (ERDÉLYI et coll.). These results provide a basis for further analysis on the mode of action of MTDQ on a molecular level. Polycyclic aromatic hydrocarbons and their decomposition products are bound covalently to the DNA during carcinogenesis (BUTY et coll. 1976). In the benzpyrene-induced experimental carcinoma of the skin the intensity of this covalent bond can be reduced by means of antioxidants (SLAGA & BRACKEN 1977).

SUMMARY

Patients with basal cell carcinoma of the skin were treated with combined MTDQ (6,6′-methylene-bis-(2,2,4-trimethyl-1,2-dihydroquinoline)) administration and irradiation. Significantly better results were obtained with a skin exposure of 2 000 R combined with MTDQ than with the same dose alone. The results were comparable to those obtained with an exposure of 4 000 R. MTDQ administration induced decrease of tissular malonaldehyde concentration and suggested the peroxide-decomposing action of the radiation sensitizer.

ZUSAMMENFASSUNG

Die kombinierte Behandlung des Basalzellenkrebs der Haut mit MTDQ (6,6′-Methylen-bis-(2,2,4-trimethyl-1,2-dihydroquinolin) und Strahlentherapie wird beschrieben. Die Ergebnisse waren nach einer kombinierten Behandlung mit 2 000 R und MTDQ signifikant besser als bei der Behandlung mit einer identischen Strahlendosis ohne MTDQ. Die Wirkung wurde auch mit den Bestahlungsergebnissen von 4 000 R verglichen. Das Verabreichen von MTDQ verringerte die Gewebskonzentration des Malonaldehyds und wies auf die peroxiddekomponierende Wirkung des strahlensensibilisierenden Mittels hin.

RÉSUMÉ

Des malades atteints de carcinome basocellulaire de la peau ont été traités par association d'administration de MTDQ (6,6′-méthylène-bis (2,2,4-triméthyl-1,2-dihydroquinoline)) et d'irradiation. On a obtenu des résultats significativement meilleurs avec une dose d'exposition cutanée de 2 000 R associée à la MTDQ qu'avec la même dose seule. Les résultats ont été comparables à ceux obtenus avec une exposition à 4 000 R. L'administration de MTDQ à entrainé une diminution de la concentration tissulaire en malonaldéhyde, ce qui fait penser que le radiosensibilisateur a une action de décomposition du peroxyde.

REFERENCES

ADAMS G. E.: Chemical radiosensitization of hypoxic cells. Brit. med. Bull. 29 (1973), 48.

BÄR V., MERCZ J., SZVOBODA J., POLLÁK Zs. és WÁTYÁS J.: Eljárás antioxidáns dihidrokinolin-származékok elöállitására. (In Hungarian.) Hungarian Patent No. 162.358. (1971).

— FORIS G., ERDÉLYI V. POLLÁK Zs. und ECKHARDT S.: Untersuchung der radiosensibilisierenden und tumorhemmenden Wirkung des als MTDQ (6,6′-Methylen-bis (2,2,4-Trimethyl-1,2-Dihydroquinolin)) bezeichneten Antioxidans. Arch. Geschwulstforsch. 5 (1975), 489.

— — — — — Biopharmacological investigation of 6,6′-methylene-bis (2,2,4-trimethyl-1,2-dihydroquinoline) (MTDQ) a radical binding antioxidant of secondary amine type. Neoplasma (Bratisl.) 24 (1977), 253.

BUTY S. G., THOMSON S. and SLAGA T. J.: The role of epidermal aryl hydrocarbon hydroxylase in the covalent binding of polycyclic hydrocarbons to DNA and its relationship to tumor initiation. Biochem. biophys. Res. Commun. 70 (1976), 1102.

EMANUEL N. M.: Kinetics and the free-radical mechanism of tumor growth. Ann. N.Y. Acad. Sci. 222 (1973), 1010.

ERDÉLYI V., BÄR V., POLLÁK ZS., FODOR J., KRALOVÁNSKI I., HINDY I., TÓTH I. and ECKHARDT S.: Clinical studies with MTDQ (6,6′-methylene-bis (2,2,4-trimethyl-1,2-dihydroquinoline)) an antioxidant with radiation sensitizing effect. To be published in Strahlentherapie.

FODOR J., POLLÁK ZS., ERDÉLYI V. and BOZSNYAI T.: (Unpublished data.)

GUTTERIDGE J. M. C.: The use of standards for malonyldialdehyde. Analyt. Biochem. 69 (1975), 518.

MASON H. S., INGRAM D. J. and ALLEN B.: The free radical property of melanins. Arch. Biochem. 86 (1960), 225.

POLLÁK ZS., RODÉ I. und BÄR V.: Beitrag zu den bio- und radiochemischen Wirkungen der Verbindung MTDQ. Strahlentherapie 154 (1978), 499.

PRYOR T.: *In:* Free radicals in biology. Vol. 1. Edited by W. A. Pryor. Academic Press, New York 1975.

— and STANLEY J. P.: A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation. J. org. Chem. 40 (1975), 3615.

RODÉ I., BÄR V., POLLÁK ZS., FARKAS J. and KISS B.: Increased radiation sensitivity in radiotherapy of malignant neoplasms. IV. Congressus Radiologicus Cechoslovacus cum Participatione Internationali. Books of abstracts, p. 148. Bratislava 1976.

— — — — — Clinical experiences with the radiation sensitizing effect of the antioxidant MTDQ. International Symposium on Radiobiologic Research, Vienna 1976. Radiobiological research and radiotherapy. Vol. 1, p. 265. IAEA, Vienna 1977.

SCHWARTZ H. M., AMBEGAONKAR S., ANTHOLINE N., KONIECZNY M. and MAILER C.: A survey of present and potential clinical applications of electron spin resonance. Ann. N.Y. Acad. Sci. 222 (1973), 989.

SHAMBERGER R. J.: Lysosomal enzyme changes in growing and regressing mammary tumors. Biochem. J. 111 (1969), 375.

— ANDREONE T. L. and WILLIS C. E.: Antioxidants in cancer. IV. Initiating activity of malonaldehyde as a carcinogen. J. nat. Cancer Inst. 53 (1974), 1771.

SLAGA T. J. and BRACKEN W. M.: The effects of antioxidants on skin tumor initiation and aryl hydrocarbon hydroxylase. Cancer Res. 37 (1977), 1631.

WATTENBERG L. W.: Inhibition of chemical carcinogen-induced pulmonary neoplasia by butylated hydroxyanisole. J. nat. Cancer Inst. 50 (1973), 1541.