TECHNICAL REPORT
RERF TR 13-82
業 績 報 告 書

PARAQUAT-INDUCED RADIOSENSITIZATION OF MAMMALIAN CELLS

哺乳動物細胞の paraquat による放射線増感作用

RICHARD C. MILLER, Ph.D.
TOSHIO FUJIKURA, M.D. 藤倉敏夫
TOSHIO HIRAOKA, M.D. 平岡敬生
HIROMI TENOU, B.Sc. 天王浩美

放影研
RERF

RADIATION EFFECTS RESEARCH FOUNDATION
財団法人 放射線影響研究所

A Cooperative Japan – United States Research Organization
日米共同研究機関

RERF TECHNICAL REPORT SERIES
放影研業績報告書集

The RERF Technical Reports provide the official bilingual statements required to meet the needs of Japanese and American staff members, consultants, and advisory groups. The Technical Report Series is not intended to supplant regular journal publication.

放影研業績報告書は，日米専門職員，顧問，諮問機関の要求に応えるための日英両語による公式報告記録である．業績報告書は通例の誌上発表論文に代わるものではない．

The Radiation Effects Research Foundation (formerly ABCC) was established in April 1975 as a private nonprofit Japanese Foundation, supported equally by the Government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.

放射線影響研究所（元ABCC）は，昭和50年4月1日に公益法人として発足したもので，その経費は日米両政府の平等分担により，日本は厚生省の補助金，米国はエネルギー省との契約に基づく米国学士院の補助金とをもって運営されている．

放影研 RERF RADIATION EFFECTS RESEARCH FOUNDATION
財団法人 放射線影響研究所

PARAQUAT-INDUCED RADIOSENSITIZATION OF MAMMALIAN CELLS

哺乳動物細胞の paraquat による放射線増感作用

RICHARD C. MILLER, Ph.D.; TOSHIO FUJIKURA, M.D. (藤倉敏夫);
TOSHIO HIRAOKA, M.D. (平岡敬生); HIROMI TENOU, B.Sc. (天王浩美)

Department of Pathology
病理部

SUMMARY

The herbicide, paraquat (methyl viologen, 1-1′ dimethyl-4, 4′-bipyridinium dichloride), stimulates the production of superoxide anion ($O_2^{\cdot-}$) in aerobic cells and therefore mimics some effects of ionizing radiation. In addition, concentrations of cellular glutathione are reduced by reaction with $O_2^{\cdot-}$. It is reported here that paraquat, toxic in its own right to aerobic cells, acts as a radiosensitizer when cells are exposed to nontoxic concentrations of the drug prior to and during irradiation.

The radiomimetic effect of paraquat, alone and in combination with X-rays, was examined. Paraquat affects aerated cells (hamster lung V79 cells) in a dose-dependent manner. Doses in excess of 1 mM for two hours cause significant cell killing. In combination with radiation, sublethal doses of paraquat, given for two hours prior to irradiation, enhance the lethal effects of radiation. However, if cells are exposed to the same concentration of paraquat following irradiation, no additional lethal effect is observed. Paraquat is a useful tool to study the effects of $O_2^{\cdot-}$ and may lead to a better understanding of the mechanisms of radiation-induced energy deposition in cells.

INTRODUCTION

The herbicide, paraquat, stimulates the production of $O_2^{\cdot-}$ in aerobic cells[1-3] and therefore mimics the effects of ionizing radiation. Paraquat, toxic in its own right to aerobic cells, acts as a

要 約

除草剤, paraquat (methyl viologen, 1-1′dimethyl-4, 4′-bipyridinium dichloride) は好気性細胞中のスーパーオキサイド負イオン ($O_2^{\cdot-}$) の生成を促進し, それによって電離放射線類似効果を示す. 加えて, 細胞内グルタチオンの濃度は$O_2^{\cdot-}$との反応により減少する. 今回は, 本来好気性細胞に有毒である paraquatが, 細胞への放射線照射前又は照射中に非毒性濃度で処理されたときには放射線増感剤として作用するということを報告する.

単独及びX線と併用した paraquat の放射線様効果を研究した. Paraquat は濃度に依存して曝気細胞 (ハムスターの肺細胞V79) に作用する. 2時間処理で濃度 1 mM を超過すると, 有意な細胞の致死が起こる. 放射線と併用すると, 放射線照射の2時間前に致死量以下の paraquat を与えた場合に放射線の致死効果を高める. しかしながら, 細胞が放射線照射後に同濃度の paraquat 処理をされた場合は, 致死効果の増強は認められない. Paraquat は, $O_2^{\cdot-}$ の効果を研究するのに有用な手段であり, 細胞における放射線生成エネルギー寄与の機序をより深く理解することができるであろう.

緒 言

除草剤, paraquat は好気性細胞中の $O_2^{\cdot-}$ の生成を促進し,[1-3] それによって電離放射線類似効果を示す. 本来好気性細胞に有毒である paraquat が非毒性濃度

radiosensitizer when cells are exposed to nontoxic concentrations of the drug prior to and during irradiation. Paraquat is a useful tool in studying the mechanisms of interaction between radiation and cells and may have clinical application.

で放射線照射前又は照射中に細胞に添加処理されたときには，放射線増感剤として作用する．Paraquat は放射線と細胞の相互作用の機序を研究するのに有用な手段であり，臨床に応用できるかもしれない．

MATERIALS AND METHODS

Hamster lung V79 cells were used in these experiments. Cell cultures were maintained in minimum essential medium (MEM), supplemented with 10% heat-inactivated fetal bovine serum. Cell suspensions were made from stock cells trypsinized while in log phase growth. Paraquat (methyl viologen, 1-1′ dimethyl-4, 4′bipyridinium dichloride) was dissolved in complete growth medium. Aerated cells were exposed to various concentrations of the drug for two hours at 37°C. Cell suspensions were irradiated with soft X-rays (40 kVp, 5 mA, with 0.2 mm Al external filtration and a dose rate calculated to be 468 rad/min) either immediately before or at the end of a 2-hour exposure to 1 mM of paraquat and incubated at 37°C. Paraquat exposure was terminated by serial dilution of the drug during cell plating. The final concentration of the drug was diluted by at least a factor of 500. After seven days incubation at 37°C in a 95% air 5% CO_2 humid incubator, cells were fixed in formalin and stained with Giemsa. Survival of reproductive integrity was determined by colony formation macroscopically.

材料及び方法

今回の実験ではハムスターの肺細胞V79が用いられた．細胞は10%熱不活化ウシ胎児血清を加えた最少必須培地（MEM）で培養された．細胞浮遊液は対数増殖期にある細胞をトリプシン処理で剝離して作成した．Paraquat（methyl viologen, 1-1′dimethyl-4, 4′-bipyridinium dichloride）は完全増殖培地に溶解した．曝気細胞は種々の濃度の paraquat で37°C，2時間処理された．細胞浮遊液は，軟X線照射（40kVp, 5 mA, 0.2mm Al filter, 線量率468rad/min）の前2時間（照射中）又は直後から1 mM の paraquat を添加し，37°C で2時間保温培養した．Paraquat 処理は細胞播種までの段階希釈によって終了させた．少なくとも500倍以上希釈した液の濃度を paraquat の最終濃度とする．空気95%，CO_2 5%の湿潤恒温器に入れ，37°C で7日間培養後，細胞はホルマリンで固定され，Giemsa 染色された．増殖能の指標となる生存率は，コロニー形成法で判定した．

RESULTS

The lethal effects on cells of a 2-hour exposure to paraquat in various concentrations are shown in Figure 1. Exposure of aerated cells to low concentrations of the drug resulted in little cell killing. However, a precipitous exponential drop in cell survival occurred when higher concentrations of the drug were added to the cell suspension. Whereas 1 mM of the drug had very little effect on cell survival, 2 mM reduced survival substantially.

結　果

異なる濃度の paraquat で2時間処理されたときの細胞の致死効果を図1に示した．低濃度の paraquat 処理では細胞致死効果がほとんどなかった．しかしながら，細胞浮遊液に高濃度の paraquat を加えると，細胞生存率は急激な指数的減少を示した．Paraquat 1 mM では細胞の生存にはほとんど致死的影響がなかったが，2 mM では実質的な生存減少が起こった．

The radiosensitization to X-rays of aerated cells by exposure to 1 mM paraquat for two hours is shown in Figure 2. The reciprocal of the slope, D_0 (the dose necessary to reduce survival on the straight portion of the survival curve to 37%) and n values (number of independent critical targets that must be hit per cell to produce an effect) of cells exposed to radiation alone or

1 mM の paraquat で2時間処理された曝気細胞のX線に対する放射線増感作用を図2に示した．放射線照射のみ，又は放射線照射後に paraquat 処理したときの放射線量一生存率曲線では，勾配の逆数，D_0（生存曲線の直線部分の生存率を37%に下げるのに必要な線量）と n 値（1細胞に致死的効果を生ずる

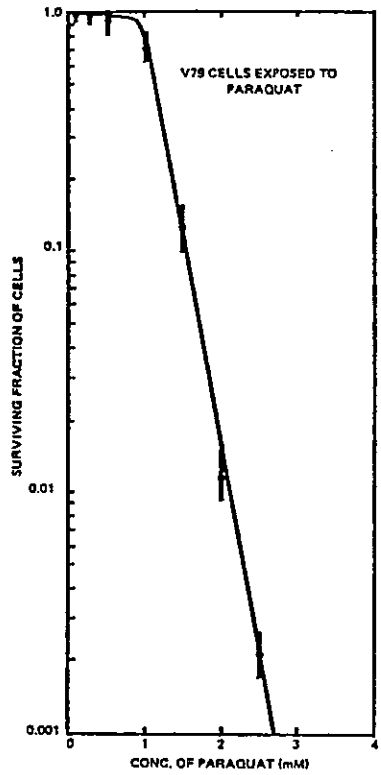

Figure 1. Cellular lethal effects after a 2-hour exposure at 37°C to various concentrations of paraquat. Experimental points represent the mean of 3 to 5 experiments ± 1 standard error of the mean.

図1　異なる濃度の paraquat で37°C で2時間処理した後の細胞致死効果．実験上の点は，3～5回の実験の平均値±1の標準誤差を示す．

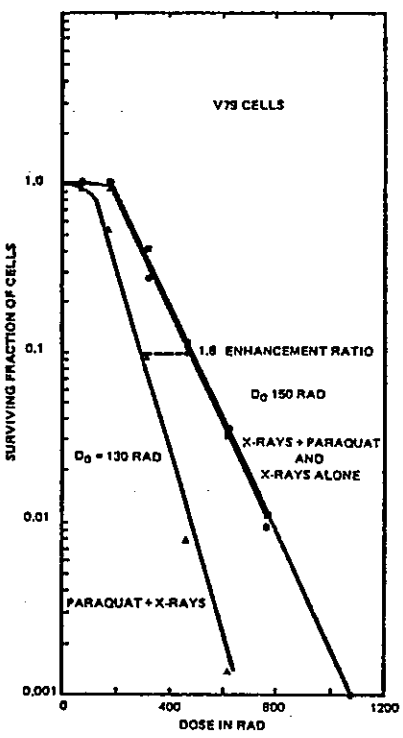

Figure 2. X-ray dose-response curves of V79 cells with or without exposure to 1 mM of paraquat for two hours. Cells were exposed to X-rays alone (●); paraquat followed by X-irradiation at the end of the 2-hour exposure (▲); or X-irradiation followed by paraquat exposure (■).

図2　1mM の paraquat で2時間処理，及び処理していない V79細胞に X 線照射した後の線量－生存率曲線．細胞が X 線にのみ照射された場合（●）；paraquat で2時間処理した後，X 線を照射した場合（▲）；X 線照射後 paraquat 処理した場合（■）．

radiation followed by paraquat, were similar. However, if cells were first exposed to paraquat for two hours prior to or during irradiation, the initial portion, or shoulder, of the survival curve was reduced. The D_0 (130 rad) for this treatment was only slightly changed from the D_0 (150 rad) of cells treated with X-rays only. At the 0.1 survival level, the enhancement ratio for cells pretreated with paraquat was 1.6. In addition to the reduction of the shoulder of the dose-effect curve, the extrapolation number (n) dropped slightly from 2.8 to 2.0.

ための標的と考えられるターゲット数）は近似していた．しかしながら，細胞が照射前又は照射中に2時間paraquat 処理された場合は，生存曲線の初めの部分，つまり肩の部分に減少が見られた．この処理を受けた場合の D_0（130rad）は X 線照射のみを受けた細胞の D_0（150rad）から多少変化しただけであった．0.1生存率レベルでは paraquat で前処理された細胞の増感率は1.6であった．線量－生存率曲線の肩の部分の減少に加えて，外挿値（n）は2.8から2.0へわずかに減少した．

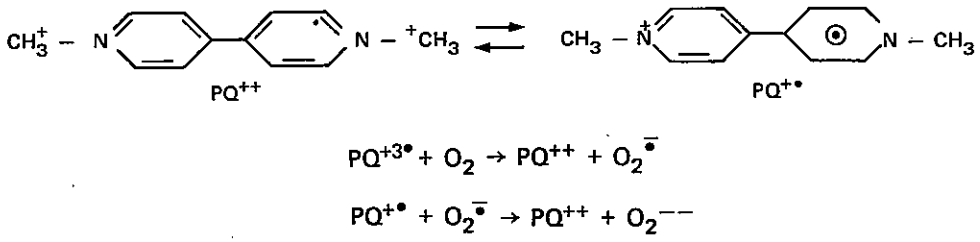

Figure 3. Schematic of the chemical structure of stable paraquat (PQ^{++}), and its oxygen sensitive form ($PQ^{+\bullet}$). Also shown is the production of oxygen radicals ($O_2^{-\bullet}$) from the interaction of paraquat and oxygen (O_2).

図3　安定 paraquat (PQ^{++}) と酸素感受性 paraquat ($PQ^{+\bullet}$) の化学構造式，また，paraquat と酸素 (O_2) の相互作用によるスーパーオキサイド ($O_2^{-\bullet}$) の生成を示す．

DISCUSSION

Microsomal stimulation in cells by paraquat promotes paraquat reduction[4] and the subsequent generation of toxic products including $O_2^{-\bullet}$ (Figure 3). These products react with DNA molecules, interfere with cell function, and result in substantial cell killing even at low concentrations of the drug. In addition, cellular glutathione (GSH) concentrations are reduced in the presence of paraquat. Gibson et al[5] have shown that when paraquat undergoes reduction-oxidation, the subsequent reduction of oxygen to $O_2^{-\bullet}$ stimulates lipid peroxidation and the resulting lipid hydroperoxides are reduced and detoxified by the oxidation of GSH. The depletion of GSH, a known scavenger of radiation-produced $O_2^{-\bullet}$, may be related to the enhanced radiosensitivity of cells exposed to nontoxic concentrations of the drug prior to irradiation.

Understanding of the combined effects of radiation and chemicals on cells is important to radiobiologists and radiotherapists alike. Modulation of radiosensitivity of cells exposed to chemicals is currently under active investigation in research laboratories and clinics.[6-11] Although paraquat in high concentrations has the potential to cause diffuse pulmonary fibrosis, its special affinity to lung tissue[12] may prove useful as an adjunct to radiotherapy.

考　察

Paraquat で細胞内のミクロソームを刺激すると paraquat の還元[4] と，$O_2^{-\bullet}$を含む毒性生成物の産生が促進される (図3)．これらの生成物は，薬品の濃度が低い場合でも，DNA 分子と反応し，細胞機能を妨害し，実質的な細胞致死を起こす．加えて，細胞内グルタチオン (GSH) 濃度は paraquat 存在下では減少する．Gibson ら[5] は paraquat が酸化還元を起こすとき，それに伴う酸素から $O_2^{-\bullet}$への還元により脂質過酸化を刺激し，結果として生ずる脂質の水素過酸化物は還元され，GSH の酸化により無毒化される．GSH は放射線生成 $O_2^{-\bullet}$の捕捉剤であることがよく知られているが，GSH の減少は照射前に非毒性濃度の paraquat で処理した細胞の放射線増感作用に関係があると思われる．

細胞への，放射線と化学物質の相互作用を理解することは放射線生物学者にとっても放射線治療専門医にとっても同様に重要である．化学物質で処理した細胞の放射線感受性の変化は現在，研究所，病院などで熱心に研究されている．[6-11] 高濃度の paraquat は瀰漫性の肺線維症を誘発するおそれがあるが，それが特に肺組織に親和性があるということは[12] 放射線治療の一助となるであろう．

Radio-activation of oxygen and the production of highly reactive O_2^- make aerobic cells up to three times more sensitive to sparsely ionizing radiation than anoxic cells. Pretreatment of cells with paraquat additionally sensitizes cells to radiation. A substantial reduction of the shoulder of the dose-response curve for cells exposed to paraquat prior to irradiation indicates a loss of repair and/or protection of critical radiosensitive targets.

酸素の放射活性化と反応性の高い O_2^- の生成により，低エネルギー電離放射線に対して，曝気細胞の感受性は，酸素欠乏細胞よりも3倍高まる．加えて，細胞をparaquatで前処理すると細胞の放射線感受性を高める．照射前にparaquat処理した細胞の線量一生存率曲線の肩の部分が実質的に減少するということは，致死放射線感受性ターゲットの修復又は防護の欠如を示す．

REFERENCES
参考文献

1. LEDWITH A: Electron transfer reaction of paraquat. In *Biochemical Mechanisms of Paraquat Toxicity.* Ed by Autor AP. New York, Academic Press, 1977. pp 21-37

2. AUTOR AP: Reduction of paraquat toxicity by superoxide dismutase. Life Sci 14:1309-19, 1974

3. HUSSAIN MZ, BHATNAGAR RS: Involvement of superoxide in the paraquat-induced enhancement of lung collagen synthesis in organ culture. Biochem Biophys Res Commun 89:71-6, 1979

4. MONTGOMERY MR: Paraquat toxicity and pulmonary superoxide dismutase: An enzymic deficiency of lung microsomes. Res Commun Chem Pathol Pharmacol 16:155-8, 1977

5. GIBSON JE, CAGEN SZ: Paraquat-induced functional changes in kidney and liver. In *Biochemical Mechanisms of Paraquat Toxicity.* Ed by Autor AP. New York, Academic Press, 1977. pp 117-36

6. DURAND RE, BROWN SM: Radiation-hypoxic cell sensitizer efficacy in multifraction exposures of an in vitro tumor model. In *Radiation Sensitizers, Vol. 5,* ed by Brady LW. Masson Pub, 1980. pp 152-63

7. HALL EJ, ASTOR M: Comparison of sensitizers in vitro. In *Radiation Sensitizers, Vol. 5,* ed by Brady LW. Masson Pub, 1980. pp 186-91

8. McNALLY NJ, DENEKAMP J, SHELDON PW, FLOCKHART IR, STEWARD FA: Radiosensitization by misonidazole (Ro 07-0582). The importance of timing and tumor concentration of sensitizer. Radiat Res 73:568-80, 1978

9. WHITE RAS, WARKMAN P, FREEDMAN LS, OWEN LN, BLEEHEN NM: The pharmaco-kinetics of misonidazole in the dog. Eur J Cancer 15:1233-42, 1979

10. DISCHE S, SAUNDERS MI, LEE ME, ADAMS GE, FLOCKHART IR: Clinical testing of the radiosensitizer Ro 07-0582: experience with multiple doses. Br J Cancer 35:567-79, 1977

11. KOGELNIK, HD: Clinical experience with misonidazole. In *Radiation Sensitizers, Vol. 5,* ed by Brady LW. Masson Pub, 1980. pp 366-73

12. ROSE MS, SMITH LL: The relevance of paraquat accumulation by tissues. In *Mechanisms of Paraquat Toxicity.* Ed by Autor AP. New York, Academic Press, 1977. pp 187-200