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Radiobiol. Radiother. **30** (1989), H. 6, 535–539
VEB Verlag Volk und Gesundheit Berlin

Strahlenbiologie

Arbeitsgruppe „Laser in der Medizin"
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The effect of Nitroimidazole and photochemotherapy on solid Ehrlich carcinomas

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Summary

The photodynamic effect of activated Hematoporphyrin in combination with the radiosensitizer Nitroimidazole was investigated. The tumor model used was the solid Ehlich carcinoma, He-Ne laser were chosen as the light source. A significant difference between the tumor masses of control animals and the tumor masses of treated animals was recorded. It is possible that the photodynamic processes are based on Type I photooxidation. Especially in the treatment of hypoxic areas of tumors the additional administration of Nitroimidazole may be important.

Key words: Nitroimidazole, Hematoporphyrin derivatives, photochemotherapy, Ehrlich carcinoma

Резюме

Эффект от нитроимидазолей и фотохимиотерапии при крупных карциномах Эрлиха

Рассматривается фотодинамический эффект от активированного гематопорфирин-деривала (HPD) в комбинации с радиочувствительным нитроимидазолом. Моделью опухоли служит крупная карцинома Эрлиха у мыши, источником излучения – He–Ne – лазер. Отмечается значительная разница между сырым весом опухолей мышей контрольной группы и весом опухолей у лечённых мышей. Возможно, что протекающие фотодинамические процессы основываются на фотооксидациях типа I. Особый интерес может представлять дополнительная аппликация нитроимидазолей при лечении гипоксических опухолевых ареалов.

Ключевые слова: нитроимидазол, гематопорфирин-деривал, фотохимиотерапия, капцинома Эрлиха

Zusammenfassung

Die Wirkung von Nitroimidazolen und Photochemotherapie auf das solide Ehrlich-Karzinom

Der photodynamische Effekt von aktivierten Hämatoporphyrin-Derivat in Kombination mit dem Radiosensitizer Nitroimidazol wurde untersucht. Als Tumormodell diente das solide Ehrlich-Karzinom. He-Le-Laser dienten als Lichtquelle. Ein signifikanter Unterschied zwischen den Tumorfeuchtmassen von Kontrolltieren und behandelten Tieren wurde beobachtet. Möglicherweise basieren die photodynamischen Prozesse auf Typ-1-Photooxydationen. Insbesondere für die Behandlung hypoxischer Tumorareale dürfte die zusätzliche Applikation von Nitroimidazolen von Interesse sein.

Schlüsselwörter: Nitroimidazol, Hämatoporphyrin-Derivat, Photochemotherapie, Ehrlich-Karzinom

Introduction

Selective photochemotherapy (PCT) of tumor bases of photodynamic processes. Both, Type I photooxidation (charge and hydrogen transfer) and Type II (energy transfer), occur.
Through activation by light, especially laser radiation, the molecules of the photosensitizer accumulated in the tumor tissue become excited (S_n). Then they cross into the long-life triplet state (T_1) by intersystem crossing transition. The T_1-state is the starting-point for Type I or Type II reactions. The result of charge transfer, especially electron transfer, is highly reactive radicals, by energy transfer singlet oxygen rices. Both radicals and singlet oxygen ($^1O_2$) lead to cytotoxic effects (*Foote* 1968 [1]).
A photosensitizer with high cytotoxic action is a mixture of different porphyrins called hematoporphyrin derivative (HpD). Singlet oxygen is thought to be the main causative agent in HpD-treatment (*Doiron* and *Gomer* 1985 [2]). Thus a large oxygen concentration in the tissue is necessary for an effective treatment.
But the O_2 concentration inside the tumor cells is often very low because of the large oxygen consumption of these cells. The diffusion radius is about 100 to 200 micrometers only (measurement on bronchial carcinoma showed a radius of 160 micrometer). Because of this tumor tissue contains a large number of hypoxic areas which can amount to 50% of solid tumors (*Magdon* 1980 [6]). The hypoxic area limit the effectiveness of PCT.
Oxygen plays an important role in radiotherapy too. In order to improve the effect of ionizing radiation by including the hypoxic cells, oxygen-mimicking compounds such as Nitroimidazole are used. Nitroimidazole are electrophilic compounds and show a high diffusion radius inside tissue. It could be shown that Nitroimidazole called "radiosensitizers" and radiation damage hypoxic tumor cells (*Magdon* 1985 [7]).
Nitroimidazole interact with activated tetrapyrroles like HpD. As a result long life porphyrin radicals and Nitroimidazole radicals arise. These show photosensitizing potentialities of Type I of photodynamic processes. The formation of singlet oxygen is inhibited. A phototoxic effect of Nitroimidazole-HpD mixtures could be seen on proteins (*Bazin* 1986 [1]).
No published data about the tumortoxic effects of these mixtures could be found. The aim of our in-vivo investigations was to get any information possible about the photodynamic effects of Nitroimidazole and HpD on the solid Ehrlich carcinoma in mice.

Materials and methods

Chemicals

The HpD used in these experiments was kindly provided by Dr. *P. Lotz* (HNO-Universitätsklinik Halle). The drug was diluted with isotonic NaCl solution and injected by i.p. adminstration and in a second experiment by s.c. administration. The dose was 5 mg/kg BW (body weight). There was one injection only, 24 hours before the first treatment.
The Nitroimidazole used was a Metronidazole called "TRIKOZOL®", i.p. administered 30 minutes before irradiation (200 mg/kg BW).

Animals and tumor model

The tumors were solid s.c. Ehrlich carcinomas in male ICR mice, induced by injection of 0,2 ml Ehlich ascites cells (5×10^6 cells/ml). First treatment was given 6 days after the administration of the cell suspension.
The mice were fed ad libitum with standard pellets and had permanent access to water. They were housed in plastic cages, temperature 22 °C to 25 °C. During treatment the mice were anaesthetized (Rompun®, Ursotamin®, NaCl).

Laser

The light source was selected taking into consideration the absorption spectrum of HpD and the tissue transmission. Therefore a wavelength in the red spectral range was used. Two He-Ne lasers (HNA 188;

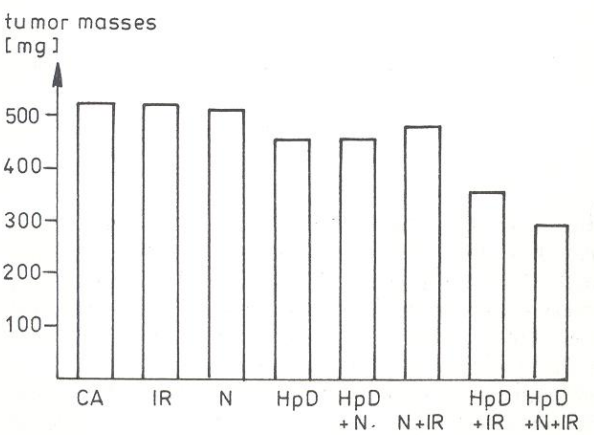
VEB Carl Zeiss JENA) were chosen as the light source emitting radiation at 632.8 nm. Each beam was coupled to a 200 μm step-index optical fibre. The fibre tips were fixed so that the laser light illuminated the tumor completely. The light intensity was 80 mW, the illuminated area about 1 cm^2.

Treatment

The tumors were irradiated twice for 30 minutes at intervals of 48 hours. The animals were divided into different groups kept under the same conditions. The first group (CA) consisted of animals without any drug administration and without laser irradiation, the second group (IR) received laser irradiation only. The group (N) received Nitroimidazole injection, the group (HpD) an administration of HpD only, whilst (HpD + N) received both. The other groups were treated with drug injection and laser treatment. The mice were killed 10 days (i.p administration) and 20 days (s.c. administration) after treatment and the weights of the tumors were determined.

Results

During irradiation the solid Ehrlich carcinomas were found to be in an expansive growth state in which first necrosis were obtained histologically. A large number of hypoxic areas can be assumed. Figure 1 and Figure 2 show the determined tumor weights (average mass) of the different animal groups. In the Table, the numbers of animals and normalized weights are listed.



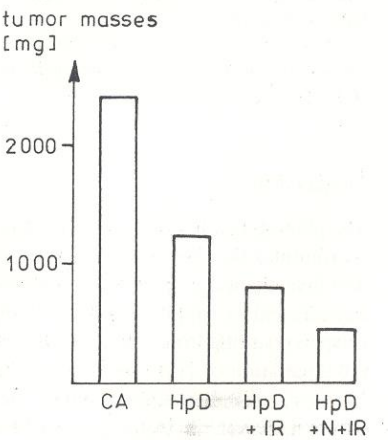


Fig. 1. Tumor masses after treatment (i.p. administration)
Tumorfeuchtmassen nach Behandlung (i.p. Applikation)

Рис. 1. Влажные массы опухоли после лечения (внутрибрюшинное применение)

Fig. 2. Tumor masses after treatment (s.c. administration)
Tumorfeuchtmassen nach Behandlung (s.c. Applikation)

Рис. 2. Влажные массы опухоли после лечения (подкожное применение)

It shows that the application of Nitroimidazole alone has no effect on the tumor growth. Also there is no detecable influence as a result of laser irradiation alone. The effect of HpD alone without irradiation on the tumor growth corresponds to the results of other PCT-experiments (*König* et al. 1988 [4], *König* et al. 1987 [5]). In our own cell treatments (human lymphocytes) we could find morphological damage caused by HpD concentrations of about 20 μg/ml without any light treatment. In other HpD-investigations (*Supino* 1986 [8]) about the effects on erythroleukemia celle there were recorded functional damages for concentrations above 1 μg/ml and morphological and functional damages for concentrations higher than

20 μg/ml without any light treatment. HpD and laser irradiation increase the cytotoxic effect as shown in the figure. Thermally induced necrosis can be excluded as the temperature increases by less than 3 K during laser irradiation. Additional administration of Nitroimidazole to the treatment seems to increase the PCT effect. However there is a significant difference between both groups (HpD + IR, HpD + Nitroimidazole + IR) in the experiment with s.c. administration only. Statistic: After a comparison of standard deviations (around 30%) by the F-test, the t-Test was used. A significant difference ($P = 0.05$) between the weights of the control group and the group with HpD, Nitroimidazole and light treatment can be obtained. There are no significant differences ($P = 0.05$) between the groups with control animals (CA), irradiation group (IR) and the group with Nitroimidazole (N).

Table
Normalized tumor masses after treatment

i.p. administration			s.c. administration		
group	n	x	group	n	x
CA	11	100	CA	8	100
IR	9	99	HpO	8	50
N	9	98	HpD + IR	15	39
HpD	7	87	HpD + N + IR	8	18
HpD + N	10	87			
N + IR	6	92			
HpD + IR	10	68			
HpD + N + IR	11	56			

CA: control animals; IR: irradiation; N: Nitroimidazol; n: number of animals; x: normalized average tumor mass; HpD: Hematoporphyrin derivative

Discussion

The photodynamic effect of the photosensitizer HpD and the additional application of the radiosensitizer Nitroimidazole after activation with longwave laser irradiation on the solid Ehrlich carcinoma in mice was investigated. It's known that Nitroimidazole interact with activated porphyrin by forming longlifing radicals and by suppressing Type II photodynamics (*Bazin* 1986 [1]). This type should be the dominant process resulting from activation of HpD in tumor tissue. Because of this it is presumed that the additional administration of Nitroimidazole decreases the efficiency of the photochemotherapy.

However, this hypothesis couldn't be confirmed by our experiments, Nitroimidazole didn't reduce cytotoxic reactions. Instead, a significant difference between the tumor masses of the control groups and the tumor masses of animals with HpD, Nitroimidazole and laser treatment could be observed. The restriction of tumor growth was greater if Nitroimidazole was added to the HpD-treatment. However, the difference is significant in the experiment with s.c. administration only ($P = 0.05$, t-test).

Maybe the photodynamic effect of the combined application of Nitroimidazole and HpD is based on Type I Photooxidation. The resulting radicals in this process show longer lifetimes than singlet oxygen.

It may be more effective, especially in the treatment of tumors with a large number of hypoxic areas if Nitroimidazole are added.

Acknowledgements

The authors are grateful to Dr. *W. Dietel* and Dr. *H. Schubert* for discussions and Mr. *U. Krause* for the technical assistance.

Literatur

1. *Bazin, M.*, and *R. Santus:* Selective Type I Photooxidations in Mixtures of Porphyrines and electrophilic Nitroimidazoles. Photochem. Photobiol. **3** (1986), 235–242. – 2. *Doirin, D. R.*, and *C. J. Gomer:* Porphyrin localization and treatment of tumors. Liss, New York 1985. – 3. *Foote, C. S.:* Mechanisms of Photosensitized oxidation. Science **162** (1968), 963–970. – 4. *König, K.*, et al.: submitted for publication. – 5. *König, K., V. Bockhorn, W. Dietel* and *H. Schubert:* Photochemotherapy of animal tumors with the photosensitizer methylene blue. J. Cancer Res. Clin. Oncol. **113** (1987), 301–303. – 6. *Magdon, E.:* Zum gegenwärtigen Stand des Einsatzes von Radiosensitizern in der Strahlentherapie aus strahlenbiologischer Sicht. Radiobiol. Radiother. **21** (1980), 788–787. – 7. *Magdon, E.:* Untersuchungen zur Wirkung von 4-Nitroimidazol auf die strahlenbedingte Wachstumshemmung und Heilungsrate von Transplantationstumoren. Radiobiol. Radiother. **26** (1985), 351–358. – 8. *Supino, R., G. Dellatorre, R. Ramponi* and *G. Bottiroli:* Effects of HpD on Mouse Erythroleukemia cells in the absence of light irradiation. Chem.-Biol. Interactions **57** (1986), 285–294.

Manuscript: 11. 11. 1988

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