FROM THE INSTITUTE OF CANCER RESEARCH, THE DANISH CANCER SOCIETY, RADIUMSTATIONEN, AND THE DEPARTMENT OF CELL BIOLOGY, THE INSTITUTE OF ANATOMY, UNIVERSITY OF AARHUS, DK-8000 AARHUS, DENMARK.

RADIATION SENSITIZING EFFECT OF HEAT

K. OVERGAARD and J. OVERGAARD

In the efforts of finding means to improve the tumour-deleting effect of a certain amount of actinic energy (sensitization) a number of physical, biologic and pharmacologic factors have been analysed but not thoroughly the possible factor heat.

A considerable number of investigations—covering all steps of a scale from human tumours through experimental implanted tumours and various forms of cultured cells to special cellular organelles or enzymatic functions—have nearly unequivocally provided evidence suggesting that heat affects malignant tissues in a different and more deletory way than normal tissue.

Although a large number of attempts to cure inoculated tumours in animals by heat have been successful, no real information has been gained as to the temperature and time relations which may determine such an effect, because continuous control of the intra-tumoural temperature has either been omitted or has been technically imperfect.

No thorough investigations of the histologic reaction of heat-treated tumour tissue have been reported, and the way in which heat may affect such tissue is quite unclear. In spite of these shortcomings reports compiled from the literature are highly suggestive as to some therapeutic possibilities of a heat treatment, and

Submitted for publication 22 April 1974.

in a previous report the question of a systematic analysis was considered (OVERGAARD & OVERGAARD 1972 a).

After the development of a tolerably reliable experimental technique, giving a possibility of continuous measurement and correction of the intra-tumoural temperature, a number of mice inoculated with an anaplastic mammary carcinoma were treated. In the temperature range of 41.5 to 43.5° C a reproducible number of cures was obtained. A relationship between the temperature used and the time of application required was demonstrated.

The clinical course was described, and a special histologic and histochemical reaction of the tumour tissue was demonstrated, suggestive of the mode of reaction. The reaction was selectively bound to tumour cells, and in successful cases all tumour cells were destroyed without any visible injury to the surrounding and interjacent normal cells.

These results, obtained exclusively by heat, may probably be elaborated for the use in human clinical practice—and, in fact, some cures of human tumours obtained by a similar treatment have been reported (CAVALIERE et coll. 1967).

However, the application of heat of sufficient intensity may be difficult and may cause discomfort. Under these circumstances some previous observations were revised and analysed, which suggested the existence of a synergistic effect of the combination of heat and roentgen irradiation (OVERGAARD 1935, OVERGAARD & OKKELS 1940, OVERGAARD & OVERGAARD 1972 b).

Method

If any synergism between heat and roentgen irradiation exists, it should be expected that combined heat—roentgen (cHR) treatment with doses of both components of a size expected separately to give no or only a few cures, may result in a definite increase in the curative effect. If that be the case, a subsequent investigation of the clinical course and of the microscopic appearances of the tumour may give suggestions as to the nature of this reaction.

An anaplastic mouse mammary carcinoma (HB) originating from the C3H strain of our Institute was implanted into the flank or inguen of C3H mice weighing 20—23 g. Untreated mice with a palpable 'take' all died with a large local tumour within 3 to 6 weeks. Metastases were few.

Tumours measuring about 5 mm × 5 mm × 6—8 mm (6 to 8 days after implantation) were used. Local heat was applied to the tumour by short-wave diathermy (27.12 Mc) using the technique previously described with continuous strict control of the intra-tumoural temperature (OVERGAARD & OVERGAARD 1972 a).

Table 1

Number of cures by local heat treatment. All cures without secondary injury

	42.5°/60	42°/120	41.5°/240	41°/480
Total surviving animals	40	18	21	5
Cures	9	4	8	2
Per cent	22	22	38	40

The term heat dose (HD) signifies the combination of tumour temperature and exposure time and is designed as a fraction, the numerator showing treatment temperature in centigrades, the denominator exposure time in minutes.

The tumour field was irradiated locally by a conventional roentgen therapy unit; the rest of the animal was shielded by 4 mm Pb. The size of the irradiated field was 1 to 2 cm^2 (factors: 250 kV, 15 mA, 36 cm skin-focus distance, 400 R inc/min). The dose is expressed in R inc.

All treatments were performed with the animals in Nembutal anaesthesia, 72 mg/kg/h being given intraperitoneally.

Whereas the roentgen technique is safe and gives a homogeneous affection of all parts of tumour, the heating technique is less perfect, as many non-controllable factors in the shape and site of the tumour may influence the heat application in some parts of the tumour and may disturb the results obtained.

To secure homogeneity of the material all the individual dose groups were compiled from single, or small numbers of, cases scattered over the total period of experimentation.

All tumours deviating from the usual mode of growth were excluded, so that the age and size of treated tumours were uniform. At least three animals were selected as controls in every inoculation batch (20 to 25 mice). The homogeneity of the relevant qualities of tumour was frequently controlled during the period of investigation.

Table 2

Effect of roentgen irradiation. All cures accompanied by heavy atrophic and fibrotic alterations

No. of surviving animals	Dose in R	Cures	Per cent
12	3 200	1	8.5
80	2 400	5	6.5
17	1 600	0	0
62	1 200	0	0

Table 3

Material treated with combined heat and roentgen irradiation. Material arranged in head-groups, partly compiled of smaller series in different temperature levels with heat doses of presumably equivalence according to StHD. Series with uniform dose components compiled without regard to variations in sequence and interval. Concurrent controls of curative effect of treatment components mentioned. Results recorded in fractions: number of cures/number of surviving animals. Cure rate in per cent valid for the head group

Heat dose StHD	Specificated HD	Roentgen irradiation		
		0 R	200 R	400 R
0	0	0/800		
1/8	40.5°/120′	1/52		
	41°/60′	0/21		
	41.5°/30′	0/24		1/19
	42.5°/7.5′	0/20		
1/4	41°/120′	0/29		
	41.5°/60′	1/45		
	42°/30′	0/34		
	42.5°/15′	1/35	3/44 (7 %)	
1/2	42°/60′	1/33		
	42.5°/30′	2/38	1/32 (3 %)	
1/1	42°/120′	4/18		
	42.5°/60′	14/66	3/20	
2	42.5°/120′	4/17		

The experimental procedures killed a number of animals—either at once or within a few days. All animals that died before 7 days were excluded as the curative effect at this period is impossible to assess.

A total and lasting cure without any signs of tumour anywhere at least 6 months after treatment was considered the only objective criterion for success, all animals which did not fulfil these conditions were counted as failures. Most of the latter animals died with evident tumour growth before 6 weeks. Only very few died during the rest of the observation period without autopsic signs of tumour. All cured animals were finally killed and subjected to autopsy, and microscopy of the tumour site performed. No signs of tumour were revealed.

Preliminary experiments

Initially, the curative heat dose (cHD) was determined in the range 41 to 42.5° C (Table 1). A higher HD appeared to increase the effect only slightly. Downwards, the curative effect is not sharply defined, but one half of the cHD or less results in only very few or no cures (Table 3).

Table 3 *(cont.)*

Roentgen irradiation						
	800 R			1200 R		1600 R	
	0/31			0/70		0/81	
						11/48	
				9/68		5/34	
	18/217						
(5 %)	0/12	(8 %)			(13 %)	2/21	(17.5 %)
						6/27	
	9/60			36/153			
	4/26						
	10/43	(17.5 %)		3/8	(24 %)	7/27	(24 %)
				23/105			
	35/146	(24 %)		6/24	(22 %)	11/26	(42 %)
				9/13			
	6/19	(31 %)		1/5	(55 %)	17/26	(65 %)
						5/8	(62 %)

In a logarithmic system, the values of cHD observed form a straight line. As all these combined values of temperature and exposure time presumably are equivalent in curative effect, and as equal fractions of the different cHD evidently provoke equal alterations in the tumour tissue (independent of the temperature range), it was found convenient (in spite of the limited material) to use this line as a working unit, the standard heat dose (1 StHD), in the combination of the physical influence and the biologic effect in this respect. Possibly, it may be valid in comparing effects of HD given at different temperature levels in the actual investigation and useful in comparative investigations to other experimental tumour systems. The validity of this assumption may be confirmed in further experiments.

Microscopy of tumours treated with subcurative doses corroborated the previous findings: destructive and mitotic alterations of a varying nature were present in all cases. The number of affected cells and the severity of the affections accorded well with the dose given, independent of the actual temperature. In all cases, a number of tumour cells were unaltered, and multicentric new-growth of tumour appeared within a few days.

Table 4

cHR series treated with uniform total doses of components, but varied according to sequence of components and time of intervals. H: heat-dose, R: roentgen dose, + : interval 0—4 h, (24): interval 24 h

H	R	H+R	H (24) R	R+H	R (24) H
41.5/30	800	6/72 (8.3 %)	5/63 (8 %)	5/47 (11 %)	2/35 (5.7 %)
41.5/60	800	3/19 (16 %)		3/20 (15 %)	3/21 (14 %)
41.5/60	1 200	3/14 (21 %)	28/118 (23.5 %)		5/21 (23 %)
42/60	1 200	5/22 (23 %)	10/40 (25 %)	4/22 (18 %)	4/21 (19 %)
42.5/30	800	10/52 (19.5 %)	7/23 (30 %)	7/27 (26 %)	11/44 (25 %)

The sensitivity of the tumour to roentgen irradiation was also estimated (Table 2). In most cases, some regression of the tumour and a transitory retardation of growth occurred after irradiation, followed by newgrowth of tumour. Varying with the size of the dose applied the survival time could be prolonged 2 to 3 months or more. Severe fibrotic and atrophic alterations were present in all cured animals. No cures were obtained by doses of 1 600 R or less.

Microscopically, all these doses produced characteristic alterations as commonly described. At 1 600 R or less, a large number of mitotic irregularities were observed, accompanied by different degrading cell formations. Extensive newgrowth of tumour cells occurred, usually within 1 to 2 weeks.

Separately, all the mentioned heat and roentgen doses gave rise to distinct alterations in the tumour tissue, but by doses below the curative level only a negligible number of cures may be expected.

Results

In the special investigation, a total of 2 881 tumour-bearing animals were used. Of 1 467 animals treated with cHR 236 mice succumbed during treatment or within the next 7 days. Of 1231 mice living at least 7 days, 241 survived for 180 days or more, presenting no signs of tumour at autopsy and local microscopy, while 982 animals which died with a definite tumour (nearly all within 6 weeks after the treatment) and 8 mice which died without a demonstrable tumour before 180 days were counted as failures. The rough rate of cures was 16.5 per cent.

Moreover, 614 mice were treated with only one of the two components in order to control the efficacy. 586 died with tumours. 800 untreated tumour-bearing controls all died with tumours.

By successive local exposure of tumours to both components in stepwise varied doses in this range a considerable number of cures were obtained (Table 3).

Here the heat doses are arranged in large groups consisting of subgroups in different temperature ranges of hypothetical equivalence (according to the size of the fraction at the StHD). The validity of this arrangement may be queried. The physical roentgen doses, on the other hand, may be estimated as biologically equivalent in the respective columns.

In most cases, the heat application was followed by roentgen irradiation for half an hour to four hours, but in some cases the sequence of the components was reversed. Furthermore, in some experiments (of both variations) the interval between the exposures was prolonged to 24 h. No significant difference in the curative effect was revealed by these variations (Table 4).

After the treatment, no local or general reaction was observed. During the next few days, the tumour became firmer in consistency. The size was mostly unchanged or decreased slightly. This state may remain almost unaltered for about 2 to 3 weeks; then the tumour hardened, became smaller and, finally, totally disappeared. The skin may be unaltered during this process, but in many cases (about one third of the animals) some infiltration of the skin formed a crust over the tumour or possibly included some of its circumference. The diameter varied from 2 mm to about 1.5 cm. This crust usually persisted for a couple of months, then gradually loosened from the border leaving a plain epithelial scar, finally covered with discoloured hair.

In animals which were not cured the initial reaction was quite identical, but some newgrowth may be palpated at the border or base of the tumour, in most cases within 1 to 2 weeks. The growth of this was like that of a normal tumour, and the animal always succumbed in 3 to 6 weeks.

Extensive microscopy of tumour tissue after cHR treatment revealed some special alterations clearly deviating from the reactions known after isolated heat or roentgen applications. In all, the more hypoxic and denser central parts of the tumour, total necrosis developed within a few hours. On the other hand, a narrow peripheral brim of active A cells revealed a greatly protracted necrobiotic decay. The most characteristic feature here was—in successful cases—the total absence of all mitotic activity in tumour cells. Histochemical examinations indicated the participation of an early and vigorous lysosomal activity in the tumour cells. (A detailed report on the microscopic findings will be published in a subsequent paper.)

Discussion

The present material clearly demonstrates the possibility of curing an implanted malignant tumour by successive application of highly subcurative doses of two different tumour-destructive agents; some synergism between heat and irradiation exists.

In addition to the demonstration of this possibility, the clinical material gives only sparse information concerning the nature of this reaction.

While curative effect of the heat dose is demonstrated as low as 1/8 StHD (and in fact exists at even lower levels), the effect of doses in the size 200 to 400 R is unsafe. Transient clinical reaction of tumour was normally observed, and microscopic alterations were constant, but cures were few.

As a whole, the complex of variations of doses in Table 2 shows a tendency to enhancement of the curative effect by an increase of each factor separately or of the two factors together. However, the significance of the quantitative relations is not sufficiently clarified. In several cases, subgroups of presumably equivalent dose combinations in different temperature ranges give very concordant results; in other cases, a wide discrepancy exists. As most of this dissimilarity may be levelled out by using the totals of the main groups as standards of reference, a larger material may settle the disturbing influence of the aforementioned non-controllable (heat) factor(s).

It must be emphasized that, in all recurrent cases, almost all tumour tissue was primarily destroyed, and newgrowth started in some peripheral part of the tumour. Presumably, some deficiency in the heat application may here be the cause. Further investigations are in progress.

SELAWRY et coll. (1958) have surveyed previous literature on this subject satisfactorily: Reports of about 500 cases of human malignant tumours treated with some form of combined heat—irradiation application are published. Most reports are from the period 1910—35; the informative value is usually low. Attention may be drawn to two groups: DOUB (1935), WARREN (1935), and SHOULDERS et coll. (1942) reported well-established palliative results in about 60 far advanced cases of human malignant tumours by a general hyperthermia at about 42° C and moderate roentgen doses. No cures were claimed. Similar results obtained by intravesical heating up to 43° C were reported by COCKETT et coll. (1967) in advanced cases of bladder tumours.

WOEBER & STEIN (1963) reduced the curative roentgen dose in skin carcinomas by 33 to 45 per cent by simultaneous addition of ultrasound—heating.

By the use of high-frequency currents (in different forms) a high effect, even cures, have been claimed; no long-term investigations confirm this, and more realistic reports (KORB 1948, BIRKNER & WACHSMANN 1949) give evidence of some palliative effect, but mostly draw attention to the technical difficulties in adequate application and measurement of heat. Presumably, these obstacles have not been overcome. Mostly a low effect was obtained by use of hyperthermic immersion (DALICHO 1957, KIRSCH & SCHMIDT 1967). In addition to some in vitro experiments suggesting a heat—roentgen synergism, a moderate number of in vivo experiments have confirmed the aforementioned observations.

During recent years, CRILE (1963), ARDENNE (1969), MUCKLE & DICKSON (1973), ROBINSON et coll. (1972) have reported similar results, using an immersion technique; only the two last-mentioned controlled the intra-tumoural temperatures. Two experimental reports (CLARKE et coll. 1970, TAYLOR 1936) could not confirm such relations.

There seems to be no possibility of comparing our treatment doses and results with previous observations. In most of these, the HD is uncertain and nearly all investigations were performed in not absolutely isologous systems. Although some parallelism in action may exist, a number of extraneous factors may influence the tumour reaction. The material studied by ROBINSON et coll. is an exception; but their analysis is rather summary, giving no possibility of estimating the number of probable cures.

In the total cHR reaction, many single elements may be traced back to alterations already known in the heat or irradiation reactions. Nevertheless, in addition to the differences in the curative effect and the clinical course, the microscopic appearances indicate a mode of action which is different from that of each of the two components.

Under such circumstances it is suggested that the combined heat—roentgen effect may depend on a synergism of the actions of the two individual components.

To estimate the common validity of this reaction similar experiments have been performed in a smaller scale on 10 other tumour systems: In general the main features in the reaction was confirmed, but special conditions in the different tumours might influence the curative possibility and microscopic details.

Acknowledgement

This investigation was supported by grants from the Krista and Viggo Petersen Foundation and the Danish Cancer Society.

SUMMARY

The radiation sensitizing effect of heat is analysed. In an isologous mouse-tumour system a successive local exposition to heat and roentgen irradiation with doses of both components of a size expected separately to give no or a few cures only an evident rise in the curative results was obtained. The presence of a synergism of the factors applied is suggested. The effect obtained depends on the dose of both factors. It is independent of the sequence of the application of the two components and is not reduced by an interval of 24 h between the exposures.

ZUSAMMENFASSUNG

Der strahlensensibilisierende Effekt von Wärme wird analysiert. An einem isologen Maus-Tumor-System ergab eine aufeinander folgende lokale Exposition mit Wärme und Rönt-

genbestrahlung mit Dosen beider Komponenten, bei denen keine oder nur wenige Heilungen zu erwarten waren, einen deutlichen Anstieg der kurativen Ergebnisse. Das Vorkommen eines Synergismus der verwendeten Faktoren wird vermutet. Der erhaltene Effekt hängt von der Dosis der beiden Faktoren ab. Er ist unabhängig von der Sequenz der Applikation der beiden Komponenten und wird nicht durch ein Intervall von 24 Stunden zwischen den Expositionen vermindert.

RÉSUMÉ

Les auteurs ont étudié l'effet radiosensibilisant de la chaleur. L'exposition locale d'un système isologue souris-tumeur à des doses de chaleur et d'irradiation roentgen qui séparément ne donneraient aucune guérison ou seulement quelques guérisons donnent lieu à une augmentation évidente des résultats curatifs. Les auteurs pensent qu'il y a une synergie de ces deux facteurs. L'effet obtenu dépend de la dose de ces deux facteurs. Il est indépendant de l'ordre dans lequel sont appliqués ces deux agents et n'est pas réduit par un intervalle de 24 heures entre les expositions.

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