AM 68/89 (Resistocell^{R}): an Important Therapeutic Agent in Advanced Malignant Epithelial Tumours

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Cancer therapy owes its most effective tools to the observations and findings of doctors working in clinics. The surgeon and cancer research scientist K.H. Bauer (1968) put it this way: "It seems appropriate to point out that in cancer research, empirical observation and experience with cancer patients, with regard to the value of the scientific insights gained, is of the same importance as experimental cancer research."
Examples of this are surgery, radiotherapy and cytostatic chemotherapy. Pure research had more the function of further investigating and safeguarding steps already taken. However, in more recent times it has turned out that this branch of research can also provide the impetus for further developments in therapy.
It is interesting to note that the origins of almost all recent approaches date back to ideas of the past decades: e.g. passive and active immunization, sulphated magnesium, selenium-eosin compounds, silver, copper, lead, gold, various dyes (Krause; Garré 1927). The concept of "endogenous defenses against cancer" gained special significance due to Paul Ehrlich (1906). For further comments see the works of Fischer-Wasels (1927); Hoepke (1952); Landsberger (1965).
Through the results of extensive research in the field of tumour immunology the existence of endogenous defense mechanisms against malignant degenerated cells must today be considered a proven fact.
In a similar manner, on the basis of long years of study, the drug "Resistocell" was developed. Basic research and clinical results both contributed equally. The basis for the development of the preparation "Resistocell" (RES) was the discovery that degranulating tissue mast cells are able to inhibit tumour growth in benzopyrene-induced sarcomas (rat) (Landsberger 1966). Of all intracellular substances this effect was attributed to an acidic glycosamine glycan.

Foundations

The basis for the development of AM 68/89 was the discovery that it is possible to inhibit the growth of experimental autochthonous tumours by paratumoural application of heparin and dextran [15]. These experiments were based primarily on two observations:
1. linear polyanionic glycans, to which heparin belongs, have the capacity to destroy tumour cells *in vitro* [17].

2. Morphological studies concerning the development and growth of uninfluenced benzopyrene-induced sarcomas found that in 6 out of 300 experimental animals the tumour had remained small. Histological examination of this phenomenon showed a mastocytosis within the tumour tissue of only these six rats, and not in any of the others. Furthermore it was observed that these mast cells had secreted the contents of their granules into the adjacent (tumour) tissue [19]. Apart from other substances such as histamine, mast cell granules contain a considerable amount of linear polyanionic glycans ([10] pp. 132-139, [2] p. 150).

To date, the development and function of the mast cell is not fully understood; it is thought to develop from connective tissue cells and is important in inflammatory processes ([4] pp. 299-301). A growth factor is also known to exist (mast cell growth factor, abbreviation: MCGF, identical with interleukin 3), which stimulates the differentiation of bone marrow cells into mast cells *in vitro* ([4] p 299/300).

We managed to induce intratumoural mastocytosis *in vivo*, in 11 out of 20 animals, by repeatedly administrating heparin and dextran [18]. Dextran was applied for its recognized mast-cell degranulating effect (see [10]). The reaction produced in this way correlated with the inhibition of tumour growth mentioned above, and in some cases even with a regression (see [15]).

Starting Points for Therapeutic Application

The problems related to paratumoural injection and the strong effect of heparin on blood coagulation did not allow for the application of this type of treatment in humans. Therefore, we conducted a detailed investigation into the properties of numerous polyanionic glycans and were surprised to find that they selectively enter tumour cells and are even able to promote the entry of other substances, such as cytostatic agents [24]. At present, the importance of anionic linear polymers in dealing with the problem of tumours is the subject of research all over the world.

In parallel, we checked whether it was possible to achieve with additions a change in the pharmacological properties of the heparin-dextran mixture, with regards to its anti-tumour effect and, after extensive experiments, succeeded using a special tissue lyophilisate (see below).

Therapeutic application of intact cells or cell clusters, i.e. tissues, by transfusion or transplantation, is a common method, frequently applied and essential to contemporary medicine. To this end, not only fresh but also preserved tissues are employed. In neurosurgery for example, extensive defects of the dura, among other things, are covered with lyophilised dura ([6] pp. 19.9 f.)

Since the early Fifties, numerous renowned scientists have also been working on the implantation of homogenised,

mainly xenogenous tissues as a promising method of treatment. Different experimental models proved that tissue homogenates enhance physiological functions, particularly with regards to regeneration [7, 11, 12, 13, 25, 31, 32].
The decision to include lyophilised tissue homogenates in our research was based on an exhaustive study by K. NEUMANN on the toxicity of lyophilised xenogenous liver, heart and placenta tissue in mice, rats, guinea pigs and dogs. He summarized the results as follows:
"An acute lethal dose by intramuscular tissue injection was not established. 200 to 500 times the normal therapeutic dose was tolerated without an acute lethal effect. Therefore, the therapeutic index of the tested preparations is at least 200 to 250.
Tests for subacute toxicity after injection of 50 times the therapeutic dose in 60 rats were satisfactory.
Tests for subchronic toxicity in dogs after injection of 50 times the therapeutic single dose over an observation period of 40 days after the start of the injections, with extensive clinical and laboratory examinations carried out after injection of fetal liver, did not reveal any signs of damage to the organism.
Bearing in mind these test results and adhering to the usual evaluation criteria, we do not see any reason to assume that, given a normal therapeutic application of correctly prepared tissue homogenates, tissue injections should have any toxic effect." (Neumann K. 1962 [30]).
Experimental and clinical data on the probability of provoking pathological immune responses, which in principle are possible as tissue homogenates contain foreign proteins, were available as well. These data reported that the risk was surprisingly low, in some cases even sensitization processes were not observed [14, 33, 35].

Function of a Combination Application

In the years 61/62 we carried out an extensive experimental study on the treatment of autochthonous tumours, in which we applied, apart from a cytostatic agent and a glucocorticoid, spleen and placenta homogenates. We did not find any indications of an antitumour effect of these tissues [16], but did obtain further insights into their properties, which we later used in our search for a supplement to the heparin-dextran mixture.
As we had observed that heparin, even after subcutaneous injection, is eliminated from the organism too quickly to have a sufficient antitumour effect, and that higher doses produced bleeding, we examined whether delayed resorption would be suitable for increasing the therapeutic spectrum. In this context we tested the addition of different substances with known resorption-delaying effects.

Both our experimental results, and theoretical considerations, clearly indicated inclusion of a tissue homogenate, as only this vehicle could contribute a decisive additional active component: enhancement of the regeneration capacity of cells of the connective and defense tissues. Whereas heparin and dextran treatment results in intratumoural mast cell induction and degranulation eliciting an immune response, the tissue homogenate supports those structures of the organism which are necessary for these reactions.
This cooperation on different levels could be the key to overcoming the immunological enhancement, which frequently occurs with numerous pure immunostimulants after exhaustion of the defense mechanisms. With AM 68/89 such negative effects have never been observed, in either animal experiments or in clinical application.

Characterization of the Tissue Used in AM 68/89

The tissue used in AM 68/89 is a homogenate of the so-called Wharton's jelly of the umbilical cord of unborn sheep, which has been preserved by lyophilisation. Wharton's jelly is a nerve- and capillary-free gelatinous connective tissue.
The gelatinous connective tissue belongs to the embryonal connective tissues and does not exist in the adult organism (mammals). It consists of a wide-meshed network of mesenchymal cells, at the differentiation stages of fibroblasts/fibrocytes, gelatinous ground substance and collagenous fibril bundles. Ground substance is quantitatively the predominant component. It is jelly-like and mainly consists of non-sulphated glycosamine glycans (anionic linear polymers). A lyophilised preparation contains all components, including the ground substance ([2] pp. 163-166 and 543, [34] pp. 143-145 and 149, [36] pp. 200-203 and 241-243, [37] pp. 109-112 and 117-127).
Although there are some studies [29], to date little is known about the basis of the effect of this tissue. There is a strikingly high proportion of anionic linear polymers, the many possible functions of which have been reported by numerous research groups [3, 26, 27, 38].

Effectiveness of the Combination in Experiments

Subsequent experiments confirmed that addition of the tissue homogenate provided the hoped-for efficacy, even with non-topical application. The derivative combination proved capable of reducing toxically-induced tissue damage through therapeutic or prophylactic application [5, 20, 23], to positively influence the incidence rate, course and/or frequency of metastases of induced tumours [1, 8, 21], to enhance the effectiveness of radiotherapy [9] and to cause lymphocyte stimulation [22]. A direct tumour-static effect was not established.

The effectiveness in control experiments, in which the tissue lyophilisate was administered without heparin or dextran, was considerably less. The synergism between heparin and membrane-associated growth factors [28] could in part explain the combined effect.

For references see German version.

Results of experimental Studies on the Effect of AM 68/89 (Resistocell^{R})

Abstracts of Selected Publications

Various research groups have experimentally investigated the effect of AM 68/89 (Resistocell^{R}). One particular focus of the studies was its use as an adjuvant treatment to radio- or chemotherapy. It was possible to verify in animal experiments many of the effects of AM 68/89 which had been clinically observed, e.g. the reduction of adverse side effects from the above mentioned therapies, or the enhancement of endogenous defenses.
Abstracts of some selected publications are provided, in order to give you an overview of the most important insights. (*Bibliographic references refer to German version.*)

Bause R., C.-J. Gros, A. Landsberger, H. Renner: Reduction of Radiation-Induced Tumour Incidence Through Stimulation Treatment with Lyophilised Fetal Cells; "Strahlentherapie" 159, 4, pp. 233-239 (1983)

The effect of an immunization treatment with lyophilised xenogenous fetal cells was studied in 7-month old female albino rats (Wistar strain). Tumour incidence after sublethal whole-body irradiation with 600 cGy was measured. In addition, histological examination of the spleen of each experimental animal was carried out. 600 cGy whole-body irradiation, after 3.5 to 6 months, cause a 55% tumour incidence. The resulting tumours are tubular adenocarcinomas of the thyroid gland. Two immunostimulation treatments with xenogenous lyophilised fetal cells (connective tissue or bone marrow) carried out 8 days before and 4 days after the whole-body irradiation significantly reduce tumour incidence to 10%/15% after 3.5 months or to 15%/25% after 6 months. A single stimulation before whole-body irradiation followed by one or two stimulations after whole-body irradiation does not result in significant protection from tumours. Histological examinations of the spleens from immunostimulated animals show a pronounced regeneration of the immune system with a significant increase in the number of follicles and a significant increase in the number of lymphocytes in the red splenic pulp. The possible clinical significance of immunostimulation with lyophilised xenogenous fetal cells in radiooncology is discussed.

Gerbes A.L., E. Haen, P. Schick, O. Messerschmidt: Studies on the Therapeutic Effect of Fetal Mesenchyme on Acute Radiation Sickness in Mice; "Strahlentherapie" 159, 5, pp. 296-298 (1983)

Fetal mesenchyme (Resistocell^{R}) was tested for its effectiveness in treating acute radiation damage in mice. A significant therapeutic success was achieved in the median lethal dose range (LD 70/30 with 635cGy), which is relevant for therapy, with one i.m. application of 80 mg/kg body weight applied one day after irradiation. Further trials to optimize and explain the results of the treatment are recommended.

Heinstein G., E. Enderle: Attempts to Prevent DENA-Induced Carcinomas Through Immunological Antecedence; "Cytobiol. Rev." 5, 3, pp. 135-143 (1981)

120 white female Wistar rats with a median body weight of 120 g were divided into four groups. For a period of 180 days, the animals in groups II to IV received the carcinogen N-nitroso-diethylamine, at a dosage of 3 mg/kg body weight per day, in their drinking water.
The animals of group I remained untreated, those in group II received a "Resistocell" implantation before application of the carcinogen, animals in group III had "Resistocell" implanted only after the development of macroscopically visible diethylnitrosamine-induced primary liver cancer, and animals in group IV were treated only with the carcinogenic agent. The main criteria for the study were survival rate and the appearance of pulmonary metastases.
Group II, which had received "Resistocell" before the induction of cancer with diethylnitrosamine, had the highest median survival time of 406 days, compared to group III with a median of 366 days and group IV with a median of 362 days. The rates of pulmonary metastases were 4/23 (17,4%) and 3/16 (18,7%), in groups II and III, respectively, far below the value of 19/25 (76%) found in group IV. Thus it was possible to obtain optimal results using a combined phrophylactic therapeutic injection-implantation of fetal mesenchyme. An indication for the implantation of fetal antigens in oncotherapy may be as a prophylaxis against metastases during the pre- and postoperative phases of surgical oncotherapy.

Kärcher K.H.: Influence of Immunomodulating Substances on the Growth of Transplanted Animal Tumours Following Exposure to Ionizing Radiation; "Cytobiol. Rev." 12, 4, pp. 112-116 (1988)

Application of "Resistocell" in combination with radiotherapy was studied in an animal model. The growth curves of transplanted tumours (plasmocytom X55) were compared in groups comprised of 10 CH3 mice each. Tumour size was 50 mm^3 on the seventh day.
Group A was not treated (control), group B received "Resistocell" (2 mg in 0.2 ml suspension, intraperitoneal), groups C and D were irradiated (3x400 rad, 200 kV X-ray, 0.5 mm copper filter, 10 mA, short distance). After irradiation group D received "Resistocell" in the above mentioned dosage. Groups E and F each received 2 irradiation treatments, as described above, and group F was additionally treated twice with "Resistocell".
"Resistocell" on its own, at the given dose, does not cause a significant change of the growth curves of the transplanted tumours. Fractionalized irradiation temporarily inhibited tumour growth and caused a slight regression. However the tumours started to grow again later.
One or two administrations of "Resistocell" in combination with radiotherapy caused permanent regression of the tumours. Inhibition of new growth or complete regression were statistically significant to the end of the observation period.

Landberger A.: Experimental Results in Tumour Immunotherapy; "Die Heilkunst" 92, 5, pp. 237-243 (1979)

Results in oncotherapy force us to reconsider and search for new therapeutic methods. In this way endogenous defense mechanisms against cancer have come to play a central role in research, through an understanding of tumour immunotherapy.
Basic research led to the recognition of endogenous defenses against cancer, which implied the possibility of stimulating the immune system as an adjuvant therapy.
Animal experiments showed a shorter survival time for animals with benzopyrene-induced sarcomas, due to a blockade of defense (CCl4), compared to untreated controls.
Another experiment showed a significantly longer survival time of tumour-bearing animals with immunostimulation (with "Resistocell").
In a lymphocyte transformation test using H3-thymidine we compared the mitogen phythaemagglutinin (PHA) with "Resistocell". The test showed that "Resistocell" was three times more effective than PHA with regards to cellular stimulation.
Various animal experiments (1968-77) have proven that immunostimulation with "Resistocell" considerably delays the growth of autochthonous tumours.
Furthermore, it has been established that "Resistocell" not only stimulates but also regenerates the corresponding tissues. Therefore, even in long-term experimental application the immune system was never exhausted. The regenerative effect on both connective tissue and lymphatic tissue was verified by histological examination.

Landsberger A., M. Drautz, U. Klement, S. Wagner: Reduction of Side Effects of Cytostatic Agents Through Adjuvant Treatment with Resistocell^{R}; "Cytobiol. Rev." 10, 3, pp. 140-145 (1986)

The effects of treatment with lyophilised xenogenous fetal tissue (Resistocell^{R}) as an adjuvant to chemotherapy were studied in animal experiments using 5-6 month old female albino rats (Wistar strain). Animals were treated with Adriamycin^{R} (2.5 mg/kg body weight per week), mitomycin (0.5 mg/kg body weight per week) and endoxan (30 mg/kg body weight per week) over a period of five weeks. Through combined treatment with fetal connective tissue (50 mg/kg body weight per week, starting eight days before the initiation of cytostatic therapy) it is possible to reduce, to a statistically significant extent, damage characteristic of cytostatic treatment, including that to the spleen (reduction of the lymphocyte-generating tissue in the white splenic pulp), liver (reduction in the nuclei of liver cells from the central lobe), heart (interstitial edema caused by Adriamycin^{R}) and in the blood profile (granulocytopenia).
The possible clinical significance to better tolerance of antineoplastic chemotherapeutic agents through adjuvant therapy with lyophilised xenogenous fetal tissue is discussed.