Animal experiments play an important role at the DKFZ despite an increasing development of alternative methods. References to the use of animal cells can be found in more than 30% of all publications. Experiments are often carried out in fields like basic research, research or the test of methods for diagnostics, prophylaxis or therapy of oncology diseases.

The main part of our central service is obtaining as well as keeping laboratory animals of different species in specified conditions. Another task involves the support of animal experiments. At present (as beginning at January 2001) there are 108 authorized and 75 indicated experiments run. In addition to that, the CAL (Central Animal Laboratory) has its own scientific projects or project participations which are described in detail either below or in another place.

Our institution is headed by two veterinarians who have gained among other things qualifications in fields like laboratory animal science and microbiology. A third veterinarian supports the management of the CAL. Among our technical staff there are jobs like animal caretakers, biological laboratory assistants and biological technical assistants. The major part of the animal caretakers have even completed its apprenticeship in the CAL.

In contrast to a number of other laboratory animal keeping, do we get our experimental animals from a few national and international suppliers. The share of self-bred animals nearly only consists of transgenic mice and is in the meanwhile at approximately 66%. Our stock of animals in the CAL amounts to approximately 28,000 animals on the annual average. On the total population the share of mice is at approximately 95% and of rats at 4%. Amphibians, mastomys, guinea pigs, rabbits and fowls are kept in considerable fewer quantities. The keeping of laboratory animals is carried out in 5 barriers, 3 container units, in isolators or individually ventilated cages (IVC) and in conventional keeping.

Our laboratory animals possess a so-called SPF-status (“specific-pathogen-free”), which means that they are free from viruses, bacteria and parasites that are specific for animal species. The microbiological routine control is effected by the CAL’s own laboratory.

The CAL is able to facilitate the continuation of the education as a specialist in laboratory animal science and/or microbiology. At present there is one veterinarian in continuation of her education. For further imparting of laboratory animal studies as well as knowledge of the subject for animal experiments, the CAL has offered a 20, then 48-hour “introductory course in animal experimentation” since 1989, supported by several committed employees of the DKFZ as well as outside experts (every other year, up to a maximum of 30 participants: Individuals working towards their diploma or doctoral degrees and interested employees. In terms of contents the regulations of the Federation of European Laboratory Animal Science Associations (FELASA) category B are covered.

Further, a 2-day course unit is offered which exclusively deals with practical issues an their translation into action.
Microbiological Monitoring of Laboratory Animals and Influences of Rodent Pathogens on Animal Experiments (R0200-2)

W. Nicklas

In cooperation with: Prof. Angel Alonso, ATV, DKFZ; Axel Benner, Biostatistic Unit, DKFZ; Dr. Hans-Jürgen Busse, University of Vienna, Austria; Dr. Lothar Rink, University of Lübeck; Dr. Martin Ryll, Vet. School, Hannover

The principal duty of the working group is microbiological quality control of laboratory animals and biological materials. The aim is to provide microbiologically standardised animals for use in experimental studies at the DKFZ. The number of animals monitored has been constant during the last years. Sera of 1500-2000 animals (rats and mice) per year were tested for antibodies to 12-15 pathogens (i.e. about 30000 serological tests). A minimum of two serologic test systems is available in our laboratory for the detection of antibodies to the most relevant pathogens. Combination of results obtained by different serologic methods helps to reduce the risk of false-positive or false-negative results. In addition to serological monitoring, approx. 1000 animals per year were necropsied and monitored bacteriologically and parasitologically. Our investigations revealed that most of our colonies of barrier-housed rodents have been free of murine viruses, mycoplasmas, and parasites. However, detection of rotavirus infection and endoparasites in some of our units during the last two years stress the need of an efficient health monitoring programme for the detection of agents that have the potential to influence results of animal experiments. Therefore, the panel of agents that are detected during routine health monitoring or in cases of diseased animals is permanently adapted to the needs. For example, methods were established to detect different Helicobacter species or Pneumocystis carinii. An endemic with this opportunistic agent in a population of immunodeficient mice showed that immediate detection and elimination of pathogenic agents is an important prerequisite for the conduction of standardised animal experiments.

In addition to animals from our own colonies, animals from external sources are regularly checked by our laboratory upon arrival. The microbiological quality of animals from commercial breeders is usually in agreement with our requirements. Transgenic animals from experimental colonies, however, are frequently infected with unwanted microorganisms (including various rodent viruses). This shows that there is still a high risk of introducing pathogens with animals coming from experimental units. This risk is increasing due to the increasing world-wide exchange of genetically modified animals between research institutes.

The still ongoing monitoring of transplantable tumours and other biological material like, e.g. ES cells, shows that most samples used in our centre are free from rodent viruses. None of more than 50 lines of embryonic stem cells (ES cells) tested were contaminated with murine viruses [1]. However, contamination with lactate dehydrogenase elevating virus (LDV) was detected in several transplantable mouse tumours which, like all other biological materials, must therefore be considered as important sources of infection.

Bacteriological monitoring of mice and rats reveals that various members of the Pasteurellaceae are widespread. We regularly isolate organisms from rats and to a lesser extent from mice which are dependent on NAD (nicotinamide adenine dinucleotide) and are therefore not detectable by commonly used culture media. Like all Pasteurellaceae, they are considered to be obligate parasites due to their high and specific nutritional requirements. We frequently isolate such bacteria from pathogen-free animals coming from commercial breeders. Only little is known on the prevalence and importance of these microorganisms. We are therefore further characterising these bacteria.

Information on characteristics of all Pasteurellaceae of rodent origin (primarily Pasteurella pneumotropica) available from the literature is often contradictory. This is one reason why such organisms are frequently misidentified and not declared in health reports. We therefore defined biochemical characteristics for these bacteria and got clear criteria to distinguish between various ‘biotypes’. Surprisingly, various phenotypic groups were isolated from either mice or rats which might indicate that they are species-specific. Our studies revealed that additional hitherto uncharacterised Pasteurellaceae species exist in rodents which are therefore not detected by most diagnostic laboratories. Meanwhile, an organism provisionally called ‘Haemophilus influenzaemurium’ has been characterised in our laboratory by phenotypic and genetic methods. This organism infects mice and has been mentioned in the literature only once. It has repeatedly been isolated in our laboratory from transgenic animals originating from various experimental colonies.

Our laboratory has been involved in the organisation of a microbiological quality assurance programme since 1991. At present, about 30 labs dealing with microbiological quality control of laboratory animals from 9 European countries (Sweden, Denmark, The Netherlands, France, Switzerland, Austria, Czech Republic, Spain, Germany) are included. This programme was initiated and formerly conducted by the Rockefeller University (New York) in cooperation with our laboratory. Since January 2000 our laboratory is the sole organiser. As a part of this programme, bacterial cultures are regularly shipped to participating laboratories. The results of identification and antibiostic sensitivity testing are collected and submitted to participating labs after compilation. It is the goal of these programmes to serve animal diagnostic laboratories as an internal self-control and to determine the quality of their performance. In addition, it will contribute to an improvement of microbiological quality control and an improved quality of animals used in experiments.

Publications (* = external co-author)
**Albumin as Multidrug Carrier System in Diagnosis and Treatment of Malignant Tumors of SD Rats (R03000-3)**

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Albumin was studied as multidrug carrier system for selective fluorescence tumor detection and for selective administration of chemotherapeutic drugs in animal tumors.

Albumin labeling was performed in 1:1 molar ratio in a specific manner to preserve the biological function of Serum Albumin (SA). In a C6-glioma model (n=25) 5-amino-fluorescein labeled to albumin (AFLc-SA) was intravenously applied for fluorescence tumor detection by laser light. Brain sections were inspected 24 h after AFLc-SA administration compared to injections of unlabeled AFL. In a Walker-sarcoma model (n=70) Methotrexat labeled albumin (MTX-SA) and Aminopterin labeled albumin (APT-SA) were intravenously injected 3 times to study their efficacy and tolerability.

All albumin labeled protein conjugates demonstrated unchanged biodistribution and plasmaclearence compared to unlabeled albumin. In the C6-glioma study AFLc-SA clearly stained the tumors under laser light activation. Using laser-scanning microscopy the dye could be localized at the lysosomal compartment [1]. At the Walker-sarcoma study the tumor-cure-rate using unlabeled MTX was 55%, using MTX-SA 60% [2], using unlabeled APT 66% and using APT-SA 83%. Albumin labeled chemotherapeutic drugs unequivocally presented much lower side effects.

This design of compounds demonstrates enhanced and selective intracellular tumor uptake using SA as multidrug carrier system as basis for ongoing clinical phase-I/II and phase-II studies in fluorescence tumor detection and chemotherapy of malignant tumors [2].

Publications (* = external co-author)


**Fluorescence Diagnostics and Photodynamic Therapy of Human Squamous Cell Carcinoma in a Mouse Tumour Model (R020000-4a)**

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The most frequent malignant neoplasia of the oral cavity is the squamous cell carcinoma. Besides traditional therapy concepts as surgery, chemotherapy and radiation therapy, photodynamic therapy (PDT) is emerging as a minimal-invasive possibility with excellent esthetic and functional results in the treatment of head and neck squamous cell carcinoma since the early eighties. All clinical investigations so far suggest, that the therapeutic success mainly depends on tumor selectivity of the applied photosensitizer.

The aim of this research project is the introduction of new, more favourable sensitzers into clinical use for the therapy of the squamous cell carcinoma of the oral cavity and to optimise their therapeutical outcome.

Immunodeficient transgenic mice (strain: RAG-2) were chosen as animal model due to precoarse experiments. Tumor growth in vivo was achieved by subcutaneous injection of a human SCC cell line (XF 354), which was cultivated in vitro. Within 2-6 weeks tumors grew to sizes of up to 0.3 cm³ at left hind leg without any signs of metastasis. Thereafter photosensitzers of the first, second and third generation (Photofrin II, m-THPC, m-THPC-PEG, m-THPCnPEG, TCP-HSA, Bacteriochlorin-PEG) were applied by intraperitoneal injection. Most of these sensitzers were developed at the DKFZ by Sinn and Schrenk.

By coupling the photosensitzers to macromolecules like PEG (polyethylen glycol) or HSA (human serum albumin) a decrease of their metabolism and an increase of their tumor uptake was intended to be achieved.

The photosensitzers were applied in various drug dosages and time intervals prior to PDT followed by transdermal activation of the photosensitizer by light of substrate specific wavelength. The animals were checked every other day for any sign of therapeutic effect or side effects for up to 6 weeks (44 days). Tumor response was measured by tumor size, signs of tumor necrosis and functional disability. For an objective documentation a tissue damage score and a functional damage score was developed and used. The tumorvolume was measured transcutaneously by a calliper. Six weeks after the performance of PDT all mice were sacrificed and the treated area was checked by histological examination. As therapeutic success a macroscopic and/or microscopic tumor remission or a significant reduction of the tumor volume was defined.

The RAG-2-tumor model could be established with a tumor take rate of more than 95% [1].
Additive Intraoperative Photodynamic Therapy of Colon Cancer and Soft Tissue Sarcoma in Mice (R0200-4b)

A comparison of different photosensitizers in photodynamic therapy (PDT)

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A clinical problem in the treatment of colon cancer and soft tissue sarcoma is the high rate of tumor recurrence after resection depending on the type, stage and grade of the tumor. Radical surgical resection combined with adjuvant therapies appear to decrease tumor recurrence rate and to prolong recurrence-free survival time. Chemotherapy and intraoperative radiotherapy (IORT) as current strategies of adjuvant cancer therapy have not let to a breakthrough so far. We evaluated additive intraoperative photodynamic therapy (AIOPDT) as an alternative treatment for colon cancer and soft tissue sarcoma in a mouse tumor model to prolong recurrence-free survival time after subtotal tumor resection [1,2].

Photodynamic therapy (PDT) is based on a selective photosensitizer (PS) accumulation in tumor cells and a PS-induced tumor cell death after stimulation by laserlight. The interaction of laserlight and intratumoral PS results in a specific intracellular formation of Singulet-Oxygen with subsequent cytotoxic tumor destruction.

In this study tumor growth was induced by subcutaneous implantation of 4x10^6 CC531 colon cancer cells respectively of 6x10^6 S117 soft tissue sarcoma cells in the hind leg of immunodeficient SWISS CD 1 nu/nu mice. After reaching a tumor size of 10 mm in diameter the different PS were applied intraperitoneally into the animals. We used 0.3 mg/kg BW of mTHPC (meso-tetrahydroxyphenylchlorin) and its macromolecular compound NPC-mTHPC-PEG (Nitrophenylcarbonat-meso-tetrahydroxyphenylchlorin-Polyethylen glykol). Furthermore 5-ALA (5-Aminolevulinic acid: 200 mg/kg BW) and Photofrin II (0.3 mg/kg BW) were used for AIOPDT. After a PS-specific time of photosensitization the tumors were resected in terms of a R1/R2 situation (microscopic/macroscopic residual disease). Tissue samples of the tumors were taken for histological evaluation.

Point spectrometry to determine tissue specific PS accumulation was performed at the site of the tumor bed, center of the tumor and the overlying skin in relation to muscle tissue as reference [3]. Fluorescence intensity was measured at the maximal emission peak of mTHPC and NPC-mTHPC-PEG at 652 nm respectively to 635 nm (5-ALA) and 630 nm (Photofrin II). AIOPDT was performed on the tumor bed with an intensity of 100 mW/cm² using an Argon-Dye-Laser system. The irradiation time and the energy doses were selected for each PS (mTHPC and NPC-mTHPC-PEG: 50 sec., 5 Joule; 5-ALA and Photofrin II: 250 sec., 25 Joule). The therapeutic effect of AIOPDT was determined by recurrence-free survival time (follow-up: 6 months) and compared to control groups for colon cancer and soft tissue sarcoma treated with conventional tumor resection only.

The spectrometric data in this study demonstrated a good and homogenous intratumoral accumulation for all PS. Highest intratumoral PS accumulation was evaluated after photosensitization with NPC-mTHPC-PEG. The median recurrence-free survival time was significantly (Logrank-test) prolonged in the colon cancer groups treated with AIOPDT and mTHPC (18 days), NPC-mTHPC-PEG (18 days) and 5-ALA (16 days) compared to the control group (12 days). After AIOPDT using the PS Photofrin II no significance in recurrence-free survival (median: 9 days) was seen compared to the control group. First study results about AIOPDT using mTHPC in the treatment of S117 soft tissue sarcoma also showed a significantly (Logrank-test) prolonged recurrence-free survival (median: 103 days) compared to the control group (20 days). Nevertheless larger animal groups are essential to confirm our preliminary data concerning the treatment of soft tissue sarcoma with AIOPDT using different PS.

The main objectives, local tumor control and prolongation of recurrence-free survival were achieved in the study. AIOPDT promises to be a feasible alternative therapeutic method with the advantage of high tumor selectivity, few side effects and the unlimited possibility to repeat this procedure. Therefore the results of this experimental study serve as basis for future clinical studies of AIOPDT for intraoperative tumor bed irradiation after surgical resec-
Publications (* = external co-author)


The Activity of New Therapeutic Agents against Subspecies of Trypanosoma brucei (R02005)

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African trypanosomiasis (sleeping sickness; acute form: T. brucei rhodesiense; chronic form: T. brucei gambiense; T. brucei brucei) is a cyclical infectious disease that is listed in the priority program of the WHO (position number 6). Half of the patients are hospitalized during the second stage (meningoencephalitis) of the disease. Since the mortality in untreated patients is nearly 100%, it can be predicted that the definite cessation of production and distribution of medications that are solely effective during this stage of the disease will lead to a dramatic situation. Thus, ethical considerations and scientific interest require exploring the available huge number of anticancer drugs for their efficacy in treating or preventing sleeping sickness.

The alkylphosphocholines (APC’s, first and second generation) represent a new class of antineoplastic agents. They possess an affinity for cell membranes, influence signal transduction and cause apoptosis. The additional properties of bone marrow stimulation, antiviral and anti-leishmaniasis activity [Kuhlencord, A. et al. 1992, Antimicrobial Agents and Chemotherapy 36 (8), 1630-1634] favored these substances for use in our initial examinations [1].

The first experiments with hexadecylphosphocholine (HPC) in vivo and in vitro exhibited a significant inhibition of the infection’s course [1,2]. The concomitant administration of nonsteroidal anti-inflammatory substances (such as phenylbutazone) led to a synergistic increase in the inhibitory effect upon trypanosomes. Therefore, experiments were performed with congeners of HPC that exhibit a lower binding to serum albumin and thus a higher availability towards the parasites.

Certain metal complexes are known for their trypanocidal activity, such as platinum derivatives, as could be confirmed recently for T. b. brucei by our group [Berger, M. R., Zillmann, U. Jahrestagung der DTG, Heidelberg (Germany), Sept. 24-27 (1997), Tagungsband, P 28]. Activities on metals which are less well characterized for their antiprotozoal activity, such as complexes of molybdenum, tungsten, and bismuth were stopped.

Alternatively, coupling of metal complexes or other anti-proliferative compounds to macromolecular carriers (Human Serum Albumin, HSA) was done by Dr. H. Sinn. From a series of complexes consisting of HSA and novanxanthone, methotrexate, arsenic, acridine-yellow, aureomycin - 1 - amino - 1 - deoxy - D - sorbitol, taurine, thionine, curcumine, or 1,4 - diaminoanthracinone, the latter complex was found to be prophylactically active.

Additional cytotoxic rivanol derivatives coupled to HSA or a sugar moiety were individually and in co-exposure synergistically active (cure rate 100%, 11/11 mice).

Publications (* = external co-author)
