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 2003) there are 134 authorized and 68 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 is in between approximately 28,000 to 30,000 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 40-hour “introductory course in animal experimentations” 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 of mice and rats an their translation into action.
Microbiological monitoring of laboratory animals and influences of rodent pathogens on animal experiments

W. Nicklas

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

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 mice and rats per year were tested for antibodies to more than 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 testing, approx. 1000 animals per year were necropsied and also monitored for bacterial pathogens and for parasites. Further, molecular methods are used routinely as diagnostic tools. PCR-based methods were established to detect different Helicobacter species, Clostridium piliforme, Streptobacillus moniliformis, and Pneumocystis carinii. PCR is also used in specific cases to demonstrate the presence or absence of pathogenic viruses (e.g., MVH) in clinical samples.

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. Genetically modified animals from experimental colonies, however, are frequently infected with unwanted micro-organisms (including rodent viruses). Among the agents found in mice which were introduced into our facility from external experimental colonies were pinworms, fur mites, Pasteurellaceae, and MHV. These findings show that there is a high risk of introducing pathogens with animals coming from experimental units and emphasize the importance of comprehensive monitoring procedures. The risk of agent introduction by subclinically infected animals is even increasing due to the increasing world-wide exchange of genetically modified animals between research institutes. Also, repeated endemics with Pneumocystis carinii in immunodeficient mice showed that immediate detection of all significant agents is an essential prerequisite to avoid both the introduction of agents into our facility and the use of animals for scientific experiments that are undisturbed by infectious agents.

The still ongoing monitoring of transplantable tumours and other biological material like, e.g. embryonic stem cells (ES cells), shows that most samples used in our centre are free from rodent viruses. None of more than 50 lines of 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 passaged in animals, 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 established specific serological tests (ELISA) for several species to simplify the detection of infection with these bacteria of which some are highly fastidious and therefore difficult to culture. It is now more easily possible to test increased numbers of animals. This helps to decrease the risk of introducing these opportunistic agents by animals from external sources.

Information on characteristics of Pasteurellaceae from 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 over 2500 isolates 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 influenzae’um’ 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 10 European countries (Sweden, Denmark, The Netherlands, France, Switzerland, Austria, Czech Republic, Spain, Great Britain, 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 antibiotic 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 to an improved quality of animals used in experiments.

The work of our group resulted in participation in different working groups and the establishment of European recommendations for the health monitoring of rodents and rabbits [2].

Publications (* = external co-author)


Imaging of Lymphatic Vessels and Glands by Fluorescence and Gadolinium Marked Human Serum Albumin (R0200-3)

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The concept of primary draining lymph nodes (= sentinel lymph nodes, SLN) bases on the hypothesis that tumors are disseminating predominantly through the lymphatic system such as in mammary carcinoma or malignant melanoma.

The detection and removal seems to be predominantly important. Actually different methods of visualization SLN and the efferent draining lymphic system are used:

- Radioactively marked colloids, like ⁹⁹Tc-Albumin
- Patentblue for the direct intraoperational imaging of lymphatic vessel and SLN.

Since the albumin conjugates (synthesized by DKFZ) Methotrextat – Albumin (MTX-HSA) and 5-Aminofluorescein - Albumin (AFLc-HAS) were already examined in clinical phase -I/II-studies our activity was focused to the development of further albumin conjugates for diagnostic procedures of the lymphatic system. The aim of our studies was to detect the draining lymphatic vessels and lymph nodes by fluorescence- and Gadolinium-labeled HSA (Gd-HSA) [1].

Fluorescence Diagnostic:
For fluorescence imaging Tetra-Carboxyphenyl-Chlorine – HSA (TCPC-HSA) and were used in an 1:1 molar ratio. Fluorescence was activated by a Xenon lamp (D-light, Storz, Tuttingen) and the fluorescence emission was observed with a long pass filter.

After intracutanéous injection of 0.1 ml TCPC-HSA in Sprague-Dawley rats within 30 minutes all retroperitoneal lymph vessels could be visualized.

To have a more suitable animal model for primary draining lymph nodes Chinchilla rabbits were also intracutanéously injected with Freunds adjuvant (hind leg) to cause an enlargement of draining lymph nodes. Some days later an i.c. injection with TCPC-HSA was performed at the same locality. Already 30 minutes later the altered Ln. popliteus and also the Lnn. inguinales and iliacales and the connecting lymph vessels could be clearly demonstrated by fluorescence.

Magnetic Resonance Imaging (MRI):
The model of the reactively altered lymph nodes (Freunds adjuvant as immune modulator) in the rabbit also served for the detection of SLN and draining lymph vessels by use of MRI. After intracutanéous injection of 0.5 ml Gd-HSA animals were examined by MRI (T1-assessment with suppression of fat). Already 30 minutes after the intracutanéous injection the primary draining lymph vessels and the SLN at the height of the fossa poplitea could be illustrated by MRI. A follow-up MRI to the abdomen showed the further draining lymphatic vessels and lymph nodes within the peritoneum.

The concept of the primary draining lymph node (and distributing vessels) was successful in the described experiment. Whereas MRI can serve as a pre-operative diagnostic method the fluorescence optical method may be used intra-operatively for the dissection of affected lymph nodes.

In future both albumin conjugates will be tested in an animal model using tumors building metastases on the lymphogenic pathway.

Publications (* = external co-authors)

Photodynamic Therapy of Oro-Pharyngal Human Squamous Cell Carcinoma in a Mouse Model (R0200-4a)

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The most frequently occurring neoplasia in the area of the oropharynx of humans is the squamous cell carcinoma with a proportion (rate) of more than 95%.

Since 1983 - besides classical concepts of surgery, radiation therapy and chemotherapy - also the photodynamic therapy was clinically introduced for the treatment of tumors. All studies established that the success of therapy is predominantly depending on the tumor selectivity of the photosensitive substance used. This equally counts for the photodynamic therapy when used as primary therapy, palliative therapy for proceeded carcinoma and the intra-operative use to lower relapse rates.

Aim of the 3 years study was the introduction of new macro-molecular bound photosensitizers of clinical use for the therapy of human squamous cell carcinoma in the mouth cavity. This required animal experiments for the comparison of new sensitizers to known standards (Photofrin II, m-THPC).

As animal model a transgenic mouse strain (RAG II) was selected. Inoculation of tumor cells (cell-line XF 354, in vitro multiplication) was performed subcutaneously on the side of the hind leg (n=180 mice).

Within 2 to 6 weeks tumors developed without building metastases (0.03 - 0.3 cm³). As photosensitive agents photosensitizer (PS) of the first, second and third generation were chosen: Photofrin II, mTHPC, m-THPC-PEG, TCPP-HSA and Bacteriochlorin - PEG [1]. By coupling of PS to macro-molecules (i. g. PEG) the metabolism should be to prolonged and additionally the tumor selectivity by an improved uptake of the carrier (HSA) was intended. To examine the potential loss of activity due to the coupling...
Suitability of Different Photosensitizers in Photodynamic Therapy (PDT) - Additive intraoperative photodynamic therapy of colon carcinoma and soft tissue sarcoma in mice (R0200-4b)

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Main 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 no remarkable effects.

We evaluated additive intraoperative photodynamic therapy (AIOPDT) as an alternative treatment for colon cancer and soft tissue sarcoma in a mice 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.

In this study tumor growth was induced by subcutaneous implantation of 4x10⁶ CC531 colon cancer cells respectively of 6x10⁶ 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-Polyethylenglykol). Further 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 (determination of 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.

Publications: (* = external co-authors)


The spectrometric data in this study demonstrated a good and homogenous intratumoral accumulation for all PS (highest intratumoral values after sensitization by NPC-mTHPC-PEG).

The median recurrence-free survival time was significantly (Logrank-test) prolonged in the colon cancer groups treated with AIOPTD and mTHPC (18 days), NPC-mTHPC-PEG (18 days) and 5-ALA (16 days) compared to the control group (12 days). After AIOPTD 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 AIOPTD using mTHPC in the treatment of SI17 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 AIOPTD using different PS.

The main objectives, local tumor control and prolongation of recurrence-free survival were achieved in the study. AIOPTD promises to be a feasible alternative therapeutic method with the advantage of high tumor selectivity, fewer 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 AIOPTD for intraoperative tumor bed irradiation after surgical resection.

Publications (* = external co-author)


Activity of Antineoplastic Agents in Trypanosoma brucei Subspecies (R0200-5 – D0301)

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Over the last decades the rational development of agents that are active against infectious protozoa of the three subspecies of African Trypanosomiasis is stagnating. Therefore more and more antineoplastic agents have lately been investigated for a potential trypanocidal efficacy. In this context we could demonstrate a moderate, nevertheless significant activity of alkylphosphocholines (APC) on the survival time of mice (NMRI) infected with T. b. brucei (strain: STIB 920) [S. Konstantinov et al., Acta Tropica 64 (1997) 145-154; U. Zillmann et al., Acta Tropica 53 (1996) 183-187].


In a second set of experiments we investigated the activity of metal complexes, since trypanosomes display a high sensitivity against arsenicals. In this context platinum derivatives were screened that are known for their antineoplastic activities. Cis-platinum (cP), which was used as lead compound, showed remarkable trypanocidal activity in vivo, following mono-therapy as well as in combination with hexadecylphosphocholine (HPC) [Berger, M. R.; Zillmann, U.: Jahrestagung der DTG, Heidelberg (1997) Tagungsband, PZ8]. Treatment with cP alone cured 50% of the test animals as documented by survival times greater than 90 days after infection. Combination treatment of cP and HPC did not surpass the activity of cP alone.

In a third set of experiments we investigated the activity of cytotoxics linked to human serum albumin (HSA) or sugar derivatives (synthesis H. J. Sinn). Out of nine complexes investigated, only HSA-1,4-diaminoanthracinone (1 ml i.p/30 gr. mouse; 0.3 mg/ml; injection 1 day prior to the infection) was prophylactically active.

In addition, acridine-orange (Rivanol®) linked to HSA or glucosamine was investigated. In vitro only the native acridine – orange exerted minimal growth inhibition in T. b. brucei. In vivo however the complexes caused prolongation of lifespan and the physical mixture of the two agents was curative in 13/13 NMRI – mice infected with T. b. brucei.

Future studies will concentrate on mechanistic aspects such as the inhibition of nucleoside – transport in trypanosomes by flavonoids alone or coupled to HSA [Mäser et al., J. Mol. Med.. 79 (2001) 121 – 127].