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Subcutaneous soft tissue tumours at the site of implanted microchips in mice

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With 3 figures

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Summary

An experiment using 4279 CBA/J mice of two generations was carried out to investigate the influence of parental preconceptual exposure to X-ray radiation or to chemical carcinogens. Microchips were implanted subcutaneously in the dorsolateral back for unique identification of each animal. The animals were kept for lifespan under standard laboratory conditions. In 36 mice a circumscribed neoplasm occurred in the area of the implanted microchip. Females were significantly more frequently affected than male mice. An influence of age or different treatment on the s. c. tumour incidence in two mice generations could not be observed. Macroscopically, firm, pale white nodules up to 25 mm in diameter with the microchip in its center were found. Microscopically, soft tissue tumours such as fibrosarcoma and malignant fibrous histiocytoma were detected.

Introduction

Identification of laboratory animals is essential in all long-term toxicity and carcinogenicity studies. Implantable microchips are recommended as an easy, secure and durable method of identification. Conventional means of animal identification are, for example, ear notches or tags but they can get lost. Nowadays, painful procedures like toe-clipping should be avoided and tattooing may not be reliable in all (pigmented) rodents.

A new method, the implantation of microchips (SACCO 1992), was used in a two-generation study in mice.

In addition to a clear animal identification implanted microchips can simplify, for example, the body weight recording of laboratory animals. After sacrificing the animal, clear identification is still possible if the transponder is kept with its tissue.

However, the subcutaneous implantation of transponders for animal identification should not affect the animals health.

Material and methods

Parental CBA/J mice were obtained from Charles River, Germany and kept under standard laboratory conditions (room temperature 21 ± 2 °C; relative humidity 60 ± 15 %; air exchange rate 15 times/h; 12 h – 12 h light-dark cycle) in the Animal House of the Hannover Medical School, Germany.

A maximum of 3 animals of the same sex were housed per cage (males must often be separated) in Macrolon Type II cages (350 cm^2) on absorbent softwood (H 3/4, Hahn & Co., Kronsberg, Germany). The animals received the pelleted standard diet 1324 (Altromin GmbH & Co. KG, Lage, Germany) and tap water *ad libitum*.

The study consisted of four main groups. Male mice were treated (with saline, urethane, and X-rays of 2 and 1 Gy) and subsequently mated with untreated virgin females 1, 3 and 9 weeks later. The offspring of each parental group was divided into two subgroups. The main purpose of this experiment was to verify the possible effect on the progeny of a preconceptual exposure to a carcinogen of their male pa-

rents. In total, 4279 mice were involved in this study. The final results of the experiment will be reported elsewhere.

For clear animal identification microchips (BioMedic Data Systems Inc., European distributor PLEXX BV, Elst, The Netherlands) were implanted subcutaneously in all 4279 animals. The "Electronic Lab Animal Monitoring System" (ELAMS™, same distributor) including transponders and a hand – held scanner attached to a notebook was used for data recording. The battery-free implantable micro-identification device (IMI®) (2 x 12 mm) contains an encoded microchip (10-digit alpha-numeric code) and a spool, hermetically sealed in a cylindrical glass capsule coated with a polypropylene cap half the size of the capsule. The microchips, prepacked in the lumen of a sterile needle, were implanted subcutaneously and dorsolaterally in the back.

These injections were carried out in the parental mice approximately 16–19 weeks of age. Microchips were implanted in the offspring at the age of 8 weeks. For weekly body weight control, the implanted microchips were scanned and after identifying the chip of the animal, the weight was transmitted automatically from the scale (Sartorius) to the notebook.

The mice were observed once daily for moribundity and mortality. Moribund animals were sacrificed by CO_2 and autopsied completely. All tissues were fixed in 4 % buffered formalin before sections were stained routinely with haematoxylin and eosin (H & E). Due to the large number of mice used in this experiment, histological examination was performed only for the target organs lung and liver and for every macroscopically remarkable tissue.

Results

During the lifespan of the mice, 1.5 % of the implanted microchips had to be substituted by new transponders. Either they had ceased to function after a maximum of 6 months in the animals and were still palpable under the skin or the microchips were lost by the animals and found in the softwood of the cages. These losses occurred mostly in the first two days but also 7 months after implantation.

In this lifetime experiment, 36 animals (0.8 %) developed a macroscopically circumscribed neoplasm at the implantation site. Contrary to sex, the different generation and treatment groups showed no influence on tumour incidence.

1.2 % of the females and 0.5 % of the males developed tumours in the chip implantation area. Female mice (27/2270) were significantly (Fisher's exact test $p < 0.01$) more frequently affected than males (9/2009). An influence of the s. c. neoplasms on lifetime was not detectable.

The lowest lifetime of soft tissue tumour-bearing male mice was 53 weeks (chip implantation at 8 weeks of age), the youngest female mouse developing tumours in the chip area was sacrificed 47 weeks after implantation of the microchip at the age of 55 weeks.

The general health of the s. c. tumour-bearing animals was considered normal, no clinical symptoms except the nodule on their back were shown. Macroscopically, firm nodules up to 25 mm in diameter with the microchip in the center were found (fig. 1). The cut surface was homogeneously pale, white-grey and often showed haemorrhagic foci.

Although the neoplasms developing at the implantation site had a mixed histologic appearance, two main tumour types were identified: the majority of the tumours were fibrosarcomas composed of interlacing bundles of monomorphic spindle cells and various amounts of collagen. Most of the nuclei were elongated and vesicular and contained one or more prominent nucleoli. These fibroblast-like cells had a moderate amount of cytoplasm but the cell boundaries were ill-defined. There was obvious mitotic activity and extensive local invasion of the surrounding tissues (fig. 2). Characteristic "herring-bone" pattern sometimes adjacent to large myxomatous areas were also observed.

The other main tumour type, diagnosed as malignant fibrous histiocytoma (MFH), showed populations of histiocyte-like and fibroblastic cells with focal collagen production in a characteristic storiform or cartwheel pattern (fig. 3). Pronounced cellular pleomorphism and numerous bizarre multinucleated giant cells with usually abundant eosinophilic cytoplasm were found corresponding to the pleomorphic subtype of MFH. Zones of necrosis and high mitotic activity were further characteristics of these tumours.

Three MFHs showed a haemangiopericytoma-like pattern, while others had areas resembling malignant schwannomas.

In none of the animals were metastases of the described tumours observed.

Discussion

Evaluation of the biocompatibility of implanted microchips have to be differentiated carefully due to the various capsule materials with different shape, surface and size of the microchips used in diverse animals.

GRYS et al. (1993) have studied biocompatibility of 31 x 3 mm white crystal glass and green glass (containing iron oxide) transponders in pigs, implanted just behind the ears.

LAMBOOIJ et al. (1992) performed similar studies with polyethylene terephtalate-covered transponders. Both examinations, 5 and 6 months after implantation, showed connective tissue capsules, on average less than 1 mm in thickness, surrounding the microchips.

Examination of tissue reactions to implanted microchips in various zoo animals (BEHLERT and WILLMS 1992) or laboratory rabbits, guinea pigs, woodchucks and amphibians (MROZEK et al. 1995) also only showed connective tissue capsules.

BALL et al. (1991) evaluated microchip implantation in Sprague-Dawley rats for up to 52 weeks. No effects on normal body weight gain, food consumption or general health of these animals were observed. Histopathologic examination showed thin rims of mature fibrous connec-

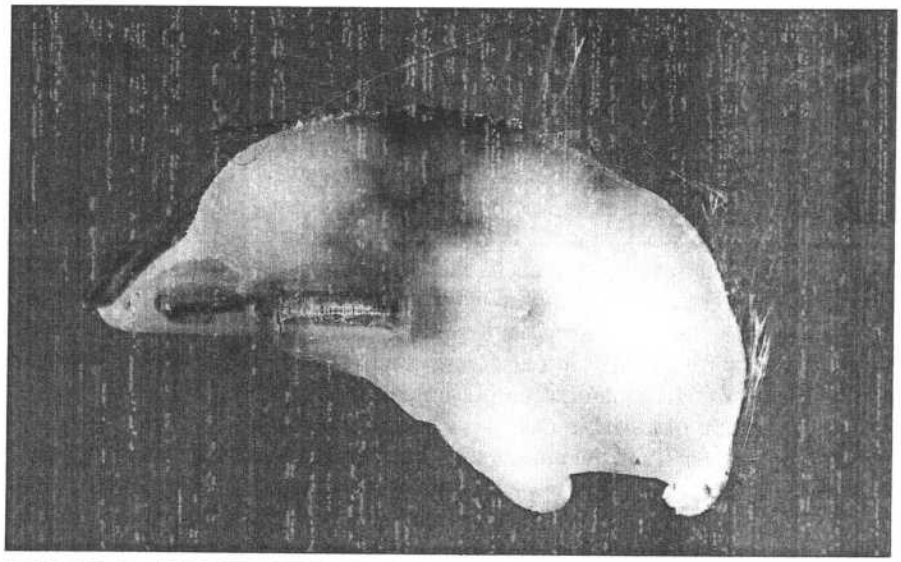

Fig. 1. Subcutaneous tumour associated with the transponder (size of the microchip 2 × 12 mm).

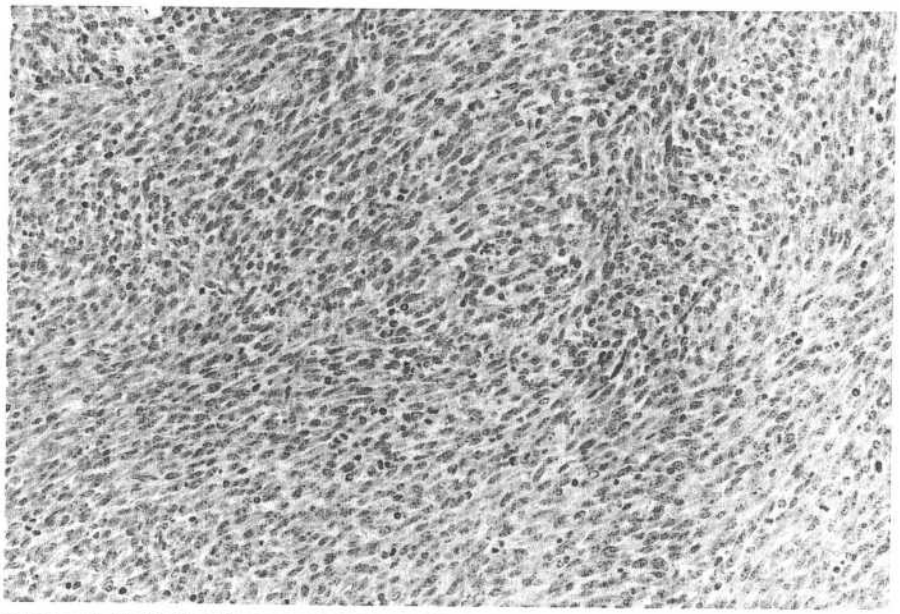

Fig. 2. Fibrosarcoma, fusiform spindle cells in charakteristic "herring-bone" pattern; H & E, × 180.

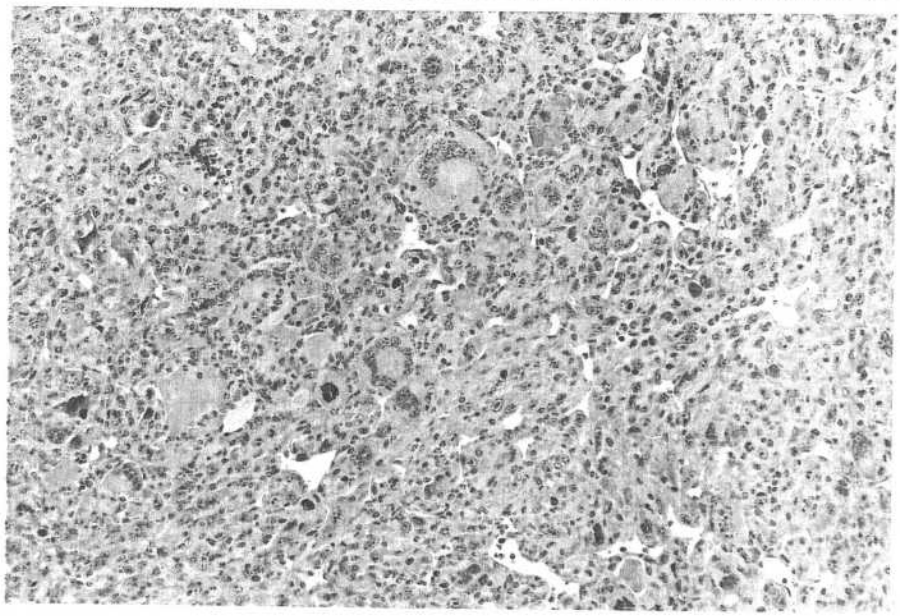

Fig. 3. Pleomorphic MFH with bizarre multinucleated giant cells; H & E, × 180.

tive tissue surrounding the implant sites, with no evidence of persistent inflammatory reaction.

Therefore, only implantation areas with macroscopic findings have been examined microscopically, so that possible pre-neoplastic lesions could have been missed.

The same glass-sealed devices with a polypropylene cap (IMI®) as used in the experiment reported here were used in a 24-month study in $B6C3F_1$ mice (RAO and EDMONDSON 1990). In seventy rodents of each sex, approximately six weeks of age, a microchip was implanted. Ten mice of each sex were necropsied at 3- and at 15 months while the remaining 100 animals were sacrificed at 24 months. Histologic examination presented a connective tissue capsule of variable thickness around most of the implants, especially in the area of the glass surface of the chips. Around the polypropylene cap of the transponder, inflammatory reactions were detected but no neoplasms observed.

These different results may be explained by the varying tumour susceptibility of inbred mouse strains and also by the low number of 140 $B6C3F_1$ mice used by RAO and EDMONDSON.

Tumours in 4279 CBA/J mice occurred only in 1.2 % of the females and in 0.5 % of the males so possibly these neoplasms at the implantation site did not occur because of the low number of animals used in their investigation.

Animal identification techniques inducing tumour development have been reported once in rats (WAALKES et al. 1987). During a 2-year study, metallic ear tags, predominantly made of nickel and copper, induced mostly osteosarcomas in about 8 % (14/168) of the Wistar rats. Almost 90 % of the rats showed tissue reactions including chronic inflammation, cartilage hyperplasia and osseous metaplasia at the tag site. Numerous observations have shown that distinct metallic compounds associated with local persistent tissue reactions may amplify tumour development, but nevertheless only two tumours were detected in 193 Wistar rats in a second study using ear tags of the same metal content. The tumour induction in this experiment seems to be due to the metallic compounds of the ear tags.

In rats, malignant fibrous histiocytomas were induced by subcutaneously implanted plastic polymers like PVC, silicon or polyhydroxyethyl methacrylate (MAEKAWA et al. 1984).

JOHNSON et al. (1973) suggested that pleomorphic tumours induced by implantation of foreign bodies (glass and plastic) in mice arose from pluripotential mesenchymal cells.

This may be an explanation for the morphologic divergency of the tumours found at the implantation site.

TAKAHASHI et al. (1990) have observed great morphological variance in subcutaneous (MFH-like sarcomas in mice. Spontaneous malignant fibrous histiocytomas showed five different growth patterns: storiform, pleomorphic, fascicular, myxoid, and hemangiopericytoma-like – similar pattern were also observed in the MFHs of the present study.

The neoplasms induced in the present investigation are clearly due to the implanted microchips.

Further information on tumorigenesis induced by microchips, e. g. experiments on their chemical components (glass and polypropylene cap), or the physical presence of the implant alone are necessary.

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