Twenty six-week exposure to 2 amino-3 methylimidazo [4,5-*f*]quinoline (IQ) does not significantly increase the incidence of tumours in HMGCR/mts1 tg579 transgenic mice

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Summary

HMGCR/mts1 tg579 transgenic mice were designed to direct the expression of metastasis-promoting mts1 (S100A4) gene to all the tissues. In order to test the usefulness of this mouse model for carcinogenicity tests shorter than that recommended by OECD guideline nr. 451, HMGCR/mts1 tg579 transgenic and C57BL/6ByA (wild type) mice (15 males and 15 females of each genotype per group) received either a control diet for 53 weeks or a control diet plus 0.03% 2-amino-3 methylimidazo[4,5-*f*]quinoline (IQ) for 26 weeks and a control diet for the remaining 27 weeks. IQ is a food mutagen with a carcinogenic effect in non-human primates and rodents. IQ is a liver carcinogen and also causes lung tumours and tumours of the forestomach in mice. Body weight gain and feed intake were decreased ($p<0.05$) during IQ feeding in IQ-dosed mice of both genotypes and sexes. The daily dose of IQ, as calculated based on the feed intake, was 43 and 46 mg/kg bw in HMGCR/mts1 tg579 transgenic males and females, and 43 and 45 mg/kg bw in C57BL/6ByA males and females. The survival rates of HMGCR/mts1 tg579 transgenic mice were 100% for males and 93% for females in the control group, and 93% for both sexes in the IQ-treated group. The survival rates of C57BL/6ByA mice of both sexes were 100% in the control group and 93% in the IQ-treated group. Non-neoplastic lesions were found in all groups, except for HMGCR/mts1 tg579 transgenic control males, primary in the liver and were of various type but of single incidence with not statistically significant difference between controls and IQ-treated groups of both genotypes and sexes. Pre-neoplastic lesions were observed preferentially in the liver in IQ-treated HMGCR/mts1 tg579 transgenic animals of both sexes. The total number of animals with tumours was: in HMGCR/mts1 tg579 transgenic mice: males - 0/15 and 3/15, females - 2/14 and 1/15 and in C57BL/6ByA: males - 0/15 and 1/15, females - 1/15 and 4/15, in control and IQ-treated animals, respectively. The primary tumours recorded in HMGCR/mts1 tg579 transgenic mice were: one pleomorphic malignant lymphoma and one histiosarcoma in the female control group, one liver hemangiosarcoma, one colon adenocarcinoma, and one malignant lymphoma/lympholytic in IQ-treated males, and one colon adenoma in IQ-treated females. The primary tumours recorded in C57BL/6ByA mice were: one histiocytic sarcoma in control females, one colon adenoma in IQ-treated males, one colon adenocarcinoma, one pleomorphic malignant lymphoma, one malignant lymphoma/lymphocytic, one thymic lymphoma, and one histiocytic sarcoma in IQ-treated females.

In conclusion, IQ feeding did not statistically significantly increase the incidence of tumours in HMGCR/mts1 tg579 transgenic and C57BL/6ByA mice in this limited bioassay. The results in HMGCR/mts1 tg579 transgenic mice obtained under current experimental conditions suggest that 53 weeks may be not a sufficient time span to demonstrate a carcinogenic potential of a test compound in this mouse model.

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Introduction

The interest in mouse models genetically modified to enhance susceptibility to carcinogenesis continues to grow *(Ashby, 2001)*. This also concerns the mouse models in which hyperproliferative or pre-neoplastic disorders marking the early stages of cellular transformation could be detected. Metastasis-promoting S100A4(mts1) protein belongs to the S100 family of Ca^{++}-binding proteins. Members of this family are implicated in a variety of activities such as cell proliferation and differentiation, cytoskeleton dynamics and apoptosis. Some members of the S100 family including *S100A4(mts1)* can be released from the cells and exhibit various physiological activities, such as stimulation of neuronal differentiation, astrocyte proliferation and modulation of activity of inflammatory cells (for review see *Donato, 2001; Heizmann, 2002*). A substantial amount of data associates expression of S100A4 with stimulation of metastasis. The *S100A4* gene was originally isolated as a gene differentially expressed in highly metastatic mouse mammary adenocarcinoma cells (*Ebralidze, 1989*). Introduction of the *S100A4(mts1)* gene into a number of nonmetastatic tumour cell lines and suppression of its activity in metastatic ones proved its involvement in metastasis formation (*Mazzucchelli, 2002*). Stimulation of tumour metastatic activity was demonstrated in two transgenic mouse models with overexpression of the *S100A4(mts1)* gene (*Ambartsumian et al, 1996; Davies et al, 1996*). In a number of human cancers the enhanced expression of *S100A4(mts1)* was associated with poor prognosis (*Davies et al, 2002; Platt-Higgins et al, 2000; Rosty et al, 2002; Yonemura et al, 2000*). All this data strongly associates the *S100A4(mts1)* gene expression with tumor progression and metastasis but the exact mechanism by which S100A4 stimulates metastasis formation remains unclear. When released into extra cellular space S100A4 stimulates angiogenesis (*Ambartsumian et al, 2001*) by promoting motility of endothelial cells. This implies that *S100A4(mts1)* could contribute to tumor progression by stimulating neovascularization of a tumour.

HMGCR/mts1 tg579 transgenic mice (indicated further as HMGCR/mts1 tg579 TR mice) were designed to direct the expression of metastasis-promoting mts1 (S100A4) gene to all the tissues. The Mts1(S100A4) gene sequences were placed under the control of ubiquitous promoter of hydroximethylglutaril Co-A reductase gene. Tissue-specific post-translational down-regulation of the Mts1(S100A4) gene product was characteristic for these animals (*Ambartsumian et al, 1998*). In old transgenic animals haemangioma in the liver and stenosis in the lungs were detected in 5-10% of cases (*Ambartsumian et. al., 2001 and our unpublished data*). Both pathological changes were characterized by hyperproliferation of endothelial and smooth muscle cells. The level of Mts1(S100A4) protein in the blood of aged transgenic animals was enhanced compared to wild type. Induction of hyper-proliferative disorders that was correlated with up-regulation of Mts1(S100A4) protein in aged transgenic animals allowed us to assume that this model could be useful for assessment of tumourigenic potential of carcinogenic agents. It was of interest to test whether the carcinogenic potential of a compound could be detected in HMGCR-mts1/tg 579 mice after a shorter period than that recommended by OECD guideline no. 451 (*OECD, 1981*) for carcinogenicity studies. 2-amino-3 methylimidazo[4,5-*f*]quinoline (IQ) was chosen as a test compound. IQ is one of 19 heterocyclic amines formed during the cooking of fish, fowl, pork, and beef and isolated as mutagens using the Ames *Salmonella typhimurium* assay (*Sugimura, 1985; Felton & Knize, 1990*). IQ administered orally by gavage to nonhuman primates induced hepatocellular carcinomas for which the average latent period was 43 and 60 months depending on dose (20 or 10 mg/kg respectively) (*Adamson et al, 1994*). IQ in dietary concentration of 0.03% induced tumours of the liver, lung, and forestomach in mice (*Oghaki et al, 1984*) and of small and large intestine, mammary gland, liver, oral cavity, and Zymbal gland in rats (*Takayama et al, 1984*)

in carcinogenicity studies for which the duration was 96 and 72 weeks respectively. Furthermore, a carcinogenic effect of IQ was demonstrated in rats exposed orally by gavage (dose 0.4 mmol/kg equal to 79.3 mg/kg) during 31 weeks and observed for a total of 52 weeks (*Tanaka et al, 1985*). However, the incidence of tumours in mice kept on a diet with 0.03% IQ during 26 weeks and observed for a total of 40 weeks was not significantly increased (*Sørensen et al, 1996*). Thus it is yet to be determined whether a time-limited exposure to IQ is carcinogenic.

The aim of this paper is to report the pathological findings in HMGCR/mts1 tg579 TR (and non-transgenic) mice, which earlier in life received a diet with 0.03% IQ for 26 weeks in order to contribute to the database on pathology of these transgenic mice and to the limited knowledge about short-term carcinogenic effect of IQ in mice.

Materials and Methods

Test compound and diets

Synthetic 2 amino-3 methylimidazo[4,5-*f*]quinoline (IQ) was obtained from the National Cancer Institute (USA). The pelleted diet Altromin 1314 (control diet) and this diet with added 0.03% IQ were obtained from Altromin GmbH u., Co KG, Lage, Germany.

Animals, housing and clinical observations

Thirty male and 30 female HMGCR/mts1 tg579 TR mice and 30 male and 30 female C57BL/6ByA mice (a wild type for the transgenic mice), 14 weeks of age were used. The initial body weight of HMGCR/mts1 tg579 TR mice was 25.9 ± 2.2 g (males) and 20.4 ± 1.0 g (females) and of C57BL/6ByA mice 27.7 ± 2.2 g (males) and 21.7 ± 1.3 g (females). All mice were bred at and obtained from the Danish Cancer Society, Division of Cancer Biology. The health status of the mice was conventional. All mice were housed one male/cage (Eurostandard type II, 267 x 207 x 140 mm) and 2-3 female/cage (Eurostandard type III, 425 x 266 x 155 mm) with aspen wood bedding (Tapvei®, Finland), kept under controlled environmental conditions (temperature 21 ± 1°C, the relative humidity 55 ± 5%, light from 09.00 to 21.00, dark from 21.00 to 09.00, air changed 10 times/h) and had free access to feed and water acidified to pH 3.0 by citric acid (to prevent growth of microorganisms). Body weight and feed intake were recorded once weekly.The spillage of feed was not recorded. All mice were observed at least twice a day for any abnormalities in clinical appearance.

Experimental design

The mice were allocated to four groups, each comprising 15 males and 15 females, based on genotype and body weight. Groups I (C57BL/6ByA) and III (HMGCR/mts1 tg579 TR) received a control diet (without IQ) during 53 weeks. Groups II (C57BL/6ByA) and IV (HMGCR/mts1 tg579 TR) received a diet with 0.03% IQ during the first 26 weeks, and a control diet for the next 27 weeks. Blood samples were collected from the orbital plexus of 5 non-fasted, non-anaesthetized animals from each group and sex prior to the start of the experiment and in weeks 12 and 26. At termination blood samples were collected from these animals from the neck wound after decapitation preceded by anaesthesia by CO_2/O_2-inhalation (60%/40% v/v). After separation of serum by centrifugation the serum samples were stored at -80°C until analysis. All mice that became moribund or with markedly disturbed general condition during the study were anaesthetized by CO_2/O_2-inhalation, decapitated, bled to death by exsanguination and autopsied. On day 371, all the remaining mice (67 weeks old) were decapitated, bled to death by exsanguination and complete gross necropsy was performed on all animals. Selected organs (liver and lungs) and all macroscopically pathological lesions were fixed in 4% neutral buffered formaldehyde, and paraffin-embedded sections, 4-6 µm thick, were stained with haematoxylin and eosin for histological examination. The study and all procedures were approved by the Danish Animal Experimental Inspectorate.

Sandwich ELISA assay for the S100A4(mts1) protein

Enzyme-linked immunosorbent assay (sandwich ELISA) was used for determination of Mts1(S100A4) content in the mouse serum. Nunc-Immuno Maxisorp 96 well plates (Life technologies) were coated with 5 mg/ml of anti-S100A4(mts1) mouse monoclonal antibodies - HM4 (*Kriajevska et al, 1994*). Mouse serum was added in dilution 1:20 in 5% BSA and incubated for 1 hour at room temperature. Second antibody was 2 mg/ml of affinity purified rabbit anti- Mts1(S100A4) (*Ambartsumian et al, 1996*). The formed complexes were visualized by sequential incubation with horseradish peroxidase, coupled goat anti-rabbit IgG, and OPD chromogenic substrate (DAKO A/S, Denmark). The reaction was quantified on an ELISA reader. The sensitivity of reaction was less than 20 ng/ml. Serum from the S100A4(mts1) -/- mice was used as a negative control in these experiments.

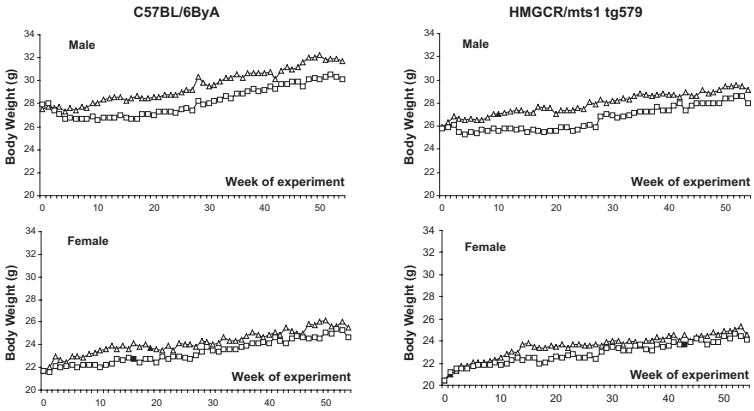
Statistical analysis

Data are presented as means ± SD where appropriate. Before being subjected to further analysis, using their standardized residuals, all data were tested for normal distribution by the Shapiro-Wilks test, and the homogeneity of variance among the groups was evaluated by judgment of standardized residuals plot. The data on body weight and feed intake showing the homogeneity of variance and normally distributed were analyzed by Student's t-test (dosed group versus control, separately for each genotype and sex). The data not showing homogeneity of variance and not normally distributed were transformed and the variance analysis was repeated. If the homogeneity of variance was still not obtained the data were subjected to the Wilcoxon test. The data on S100A4(mts1) protein content in mouse serum were compared by repeated measures analysis. Kaplan-Meier survival curves were plotted for all treatment groups for each genotype and sex and data on survival were analyzed using the lifetest procedure. The data on histopathological findings were analyzed by Fisher's exact test (one sided) for pair-wise comparison. Values of $p<0.05$ were considered statistically significant. All statistical analyses were performed using Statistical Analysis System (SAS) software (release 6.12, 1996, SAS Institute Inc. Cary, NC, USA).

Results

The initial body weights in all groups with regard to the genotype and sex were not statistically significantly different (Fig. 1). However, statistically significantly lower body weight was recorded in

Figure 1. Body weight of C57BL/6ByA (left panel) and HMGCR/mts1 tg579 TR (right panel) mice of both sexes kept on a control diet (△) or a control diet to which 0.03% IQ was added (□) for the first 26 weeks and a control diet for 27 consecutive weeks.



IQ-fed mice of both genotypes and sexes when compared to their respective controls on several occasions: in C57BL/6ByA males in weeks 9-29, 31-34, 36, 38 and 46-49, in C57BL/6BYA females in weeks 5, 7-11, 13, 15-19, 23-25, in HMGCR/mts1 tg579 TR males in weeks 3, 9-27, 29-36, 38-39 and 42, and in HMGCR/mts1 tg579 TR females in weeks 10, 13-18, 20, 23-24 and 26. At the end of IQ-feeding the body weights of IQ-treated C57BL/6BYA mice were 94% (males) and 96% (females), and of IQ-treated HMGCR/mts1 tg579 TR mice 93% (males) and 95% (females) of the respective controls. The body weight gain was statistically significantly decreased in all IQ-treated groups during the feeding of the test compound (Table 1). No difference in clinical appearance of IQ-dosed and control mice was recorded for mice of both genotypes of either sexes.

The mean relative feed intake of IQ-treated C57BL/6BYA and HMGCR/mts1 tg579 TR mice of both sexes was statistically significantly lower compared to the respective controls during the feeding IQ added diet (Table 1). After cessation of IQ feeding the only statistically significant difference in feed intake was an increased consumption of C57BL/6ByA IQ-treated female mice compared to the controls.

As seen in Table 2, the only statistically significant difference in the serum content of Mts1(S100A4) protein was a decrease recorded in IQ-treated C57BL/6BYA males when compared to the respective controls in week 26.

The survival of IQ-dosed C57BL/6ByA and HMGCR/mts1 tg579 TR mice of both sexes was not statistically significantly different compared to the respective controls (Fig. 2). All C57BL/6ByA con-

Table 1. Body weight gain, relative feed intake and dose of IQ of C57BL/6ByA and HMGCR/mts1 tg579 TR mice of both sexes kept on a control diet for 53 weeks or a control diet to which 0.03% IQ was added for the first 26 weeks and a control diet for 27 consecutive weeks.

Group	0.03% IQ	Sex	No. of mice	Body weight gain (g)		Relative feed intake (g/kg bw/day)		Dose of IQ[a] (mg/kg bw/day)
				Weeks 1-26	Weeks 27-53	Weeks 1-26	Weeks 27-53	
I. C57BL/6ByA	-	M	15	1.5 + 1.5	1.4 + 1.0	154.0 ± 10.7	143.1 ± 10.4	-
	-	F	15	1.8 + 1.2	1.2 + 1.4	164.1 ± 13.6	149.5 ± 10.9	-
II. C57BL/6ByA	+	M	15	-0.7 + 1.4***	1.8 + 1.3	144.7 ± 7.7*	148.1 ± 9.2	43.4 ± 2.3
	+	F	15	1.4 + 1.1	1.2 + 1.1	147.0 ±11.7**	162.9 ± 13.8**	44.5 ± 3.7
III. HMGCR/mts1 tg579	-	M	15	1.6 + 0.9	0.9 + 0.7	154.5 ± 10.4	148.0 ± 7.4	-
	-	F	15	2.6 + 0.9	1.1 + 1.4	169.9 ± 9.8	160.4 ± 12.9	-
IV. HMGCR/mts1 tg579	+	M	15	-0.1 + 0.9***	1.2 +0.6	143.4 ± 6.4**	152.9 ± 9.4	43.0 ± 1.9
	+	F	15	1.3 + 0.7***	1.0 + 0.7	151.6 ± 10.2***	158.7 ± 15.1	45.8 ± 3.2

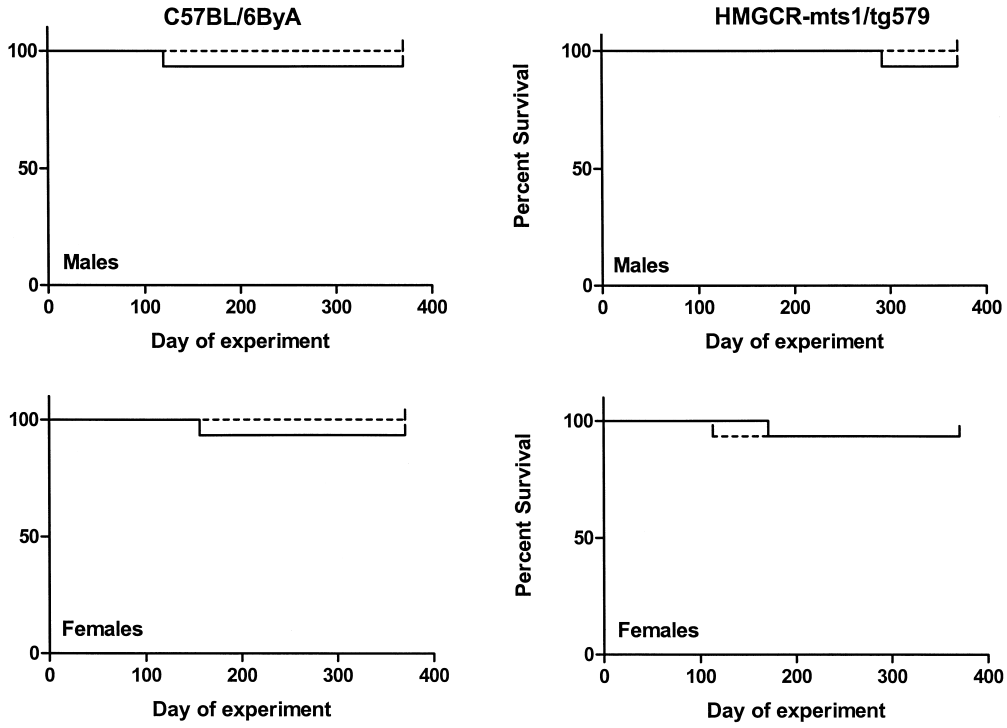
[a]: calculated based on feed intake in weeks 1-26. *: $p<0.05$, **: $p<0.01$, ***:$p<0.001$, compared with the control group, Student's *t*-test

Table 2. Serum content of Mts1(S100A4) of C57BL/6ByA and HMGCR/mts1 tg579 TR mice of both sexes kept on a control diet for 53 weeks or a control diet to which 0.03% IQ was added for the first 26 weeks and a control diet for 27 consecutive weeks.

Group	0.03% IQ	Sex	No. of mice	Week of treatment			
				0	12	26	53
I. C57BL/6ByA	-	M	5	0.252 ± 0.025	0.262 ± 0.131	0.241 ± 0.036	0.200 ± 0.036
	-	F	5	0.393 ± 0.126	0.271 ± 0.069	0.312 ± 0.092	0.245 ± 0.042
II. C57BL/6ByA	+	M	5	0.223 ± 0.026	0.272 ± 0.229	0.183 ± 0.035*	0.194 ± 0.050
	+	F	5	0.265 ± 0.118	0.208 ± 0.062	0.335 ± 0.124	0.294 ± 0.052
III. HMGCR/mts1 tg579	-	M	5	0.257 ±0.055	0.186 ± 0.052	0.206 ± 0.088	0.200 ± 0.051
	-	F	5	0.470 ± 0.216	0.300 ± 0.139	0.285 ± 0.139	0.261 ± 0.064
IV. HMGCR/mts1 tg579	+	M	5	0.233 ± 0.052	0.203 ± 0.057	0.190 ± 0.060	0.248 ± 0.097
	+	F	5	0.325 ± 0.180	0.179 ± 0.078	0.197 ± 0.038	0.231 ± 0.047

* : $p<0.05$ compared to group I.

Figure 2. Survival of C57BL/6ByA (left panel) and HMGCR/mts1 tg579 TR (right panel) mice of both sexes kept on a control diet (---) or a control diet to which 0.03% IQ was added (___) for the first 26 weeks and a control diet for 27 consecutive weeks.



trols of both sexes survived until termination. In IQ-dosed C57BL/6ByA mice one male with affected clinical condition was euthanized on day 120 and found to have a purulent bronchopneumonia, and one female, which developed a wound on the right front limb was euthanized on day 156. No tumours were found in these mice. In HMGCR/mts1 tg579 TR mice, all control males survived until termination. One control HMGCR/mts1 tg579 TR female was found dead on day 113. The cause of death was not clarified by necropsy and this animal was not included in the microscopic examination because of autolysis. In IQ-dosed HMGCR/mts1 tg579 TR mice one mouse of each sex was euthanized due to the affected clinical condition. A male euthanized on day 292 was found to have haemangiosarcoma in the liver. A female was euthanized on day 171 but the cause of the affected clinical condition was not clarified as no macroscopic changes were recorded. Non-neoplastic lesions were found in all groups except for HMGCR/mts1 tg579 TR control males (Table 3). The lesions were primarily in the liver, and were of various type but of single incidences. In C57BL/6BYA mice the changes were evenly distributed among the controls and IQ-treated males. In IQ-treated HMGCR/mts1 tg579 TR mice of both sexes the non-neoplastic lesions were more various than in the controls. Pre-neoplastic lesions were observed preferentially in the liver in IQ-treated HMGCR/mts1 tg579 TR animals of both sexes.

The control male mice of both genotypes developed no tumours during the study (Table 4). The number

Table 3. The incidence of non-neoplastic and pre-neoplastic lesions of C57BL/6ByA and HMGCR/mts1 tg579 TR mice of both sexes kept on a control diet for 53 weeks or a control diet to which 0.03% IQ was added for the first 26 weeks and a control diet for 27 consecutive weeks.

Genotype	C57BL/6ByA				HMGCR/mts1 tg579			
Sex	Males		Females		Males		Females	
Dose of IQ (%)	0	0.03	0	0.03	0	0.03	0	0.03
Liver								
Number examined	15	15	15	15	15	15	14[(a)]	15
Non-neoplastic								
Mononuclear cell infiltration	1	3	2	-	-	-	2	1
Single cell necrosis	-	1	-	-	-	1	-	2
Necrosis hepatocytes	2	-	1	-	-	1	1	1
Haematopoiesis	-	1	5	1	-	-	1	3
Mitosis increased	-	-	-	-	-	1	-	-
Pre-neoplastic								
Peliosis hepatis	1	-	-	-	-	1	-	-
Basophilic cell focus	-	-	-	-	-	1	-	1
Clear cell focus	-	-	-	-	-	-	-	1
Oval cell proliferation	-	-	-	-	-	1	-	1
Lung								
Number examined	15	15	15	15	15	15	14[(a)]	15
Non-neoplastic								
Lymphoid cell infiltration	-	-	-	-	-	1	-	-
Spleen								
Number examined[(b)]	0	0	3	0	0	0	0	0
Pre-neoplastic								
Hyperplasia lymphoid	-	-	2	-	-	-	-	-
Erythroid hyperplasia	-	-	1	-	-	-	-	-

[(a)]: One animal was excluded from microscopic examination as it was found dead and autolytic on day 113 of the study.
[(b)]: only macroscopically changed spleens were examined by microscopy.

Table 4. Incidence of primary tumours in C57BL/6ByA and HMGCR/mts1 tg579 TR mice of both sexes kept on a control diet for 53 weeks or a control diet to which 0.03% IQ was added for the first 26 weeks and a control diet for 27 consecutive weeks.

Genotype	C57BL/6ByA				HMGCR/mts1 tg579			
Sex	Males		Females		Males		Females	
Dose of IQ (%)	0	0.03	0	0.03	0	0.03	0	0.03
Total no. of mice	15	15	15	15	15	15	14[(a)]	15
Total mice with tumours	0	1	1	4	0	3	2	1
Tumour site and type								
Liver								
Haemangiosarcoma	-	-	-	-	-	1	-	-
Colon								
Adenoma	-	1	-	-	-	-	-	1
Adenocarcinoma	-	-	-	1	-	1	-	-
Haemolymphoreticular system								
Pleomorphic malignant lymphoma	-	-	-	1	-	-	1	-
Malignant lymphoma/lymphocytic	-	-	-	1	-	1	-	-
Thymic lymphoma	-	-	-	1	-	-	-	-
Histiocytic sarcoma	-	-	1	-	-	-	1	-

[(a)]: One animal was not included in microscopic examination as it was found dead and autolytic on day 113 of the study.

of mice with tumours in IQ-treated groups of both genotypes and sexes was not statistically significantly increased compared to the respective controls. The tumours were of single incidences and were recorded in haemolymphoreticular system, colon and liver.

Discussion

The dose of IQ in the present study was chosen based on the previous reports in rats (*Takayama et al, 1984; Tanaka et al; 1985*) and mice (*Ohgaki et al, 1984*). In rats ingestion of 0.03% IQ in the diet was associated with lower body weight and slightly lower feed intake in males (*Takayama et al, 1984*). In mice fed a diet with 0.03% IQ, a slightly lower body weight was reported in females only and the feed intake was not affected in both sexes (*Ohgaki et al, 1984*). In the present study, feeding a diet with 0.03% IQ was associated with a statistically significantly decreased body weight gain of mice of both genotypes and sexes compared to the respective controls. It was regarded as a consequence of a statistically significantly lower feed intake during the IQ-feeding period. This could be due to a possible toxicity of IQ and due to a lower palatability of the test diet. The recorded decreased body weight gain in the present study is in accordance with our earlier report on the effect of IQ in C57BL/6ByA and in Eμ-*pim*-1 transgenic mice (*Sørensen et al, 1996*).

In F334 rats, dietary exposure to 0.03% IQ resulted by day 300 in the following incidences of tumours in males: 45% in Zymbal gland, 22% in colon, 12.5% in small intestine, 20% in the liver, 10% in the skin, and 5% in the oral cavity, and in females: 10% in Zymbal gland and 5% in clitoral gland (*Takayama et al, 1984*). The tumour incidences of males in the same study as reported later by Ohgaki (2000) were 90% in Zymbal gland, 63% in colon, 30% in small intestine, 68% in the liver, 43% in small intestine after 55 weeks of the experimental feeding. In females the development of tumours was slower and was 68% in Zymbal gland, 50% in clitoral gland, 45% in the liver and 23% in large intestine *(Ohgaki, 2000)* after 72 weeks of experimental feeding.

In another rat study limited to one sex only, Sprague-Dawley females receiving IQ in a daily dose of 0.4 mmol/kg bw (equal to 79.3 mg/kg bw) by gavage for 31 weeks, from the age of 6 weeks and observed for a total of 52 weeks developed tumours in mammary gland, ear duct and liver with incidences of 44%, 34% and 19%, respectively (*Tanaka et al, 1985*).

In CDF_1 mice 7 weeks old at the start of the experiment and given diet containing 0.03% IQ for up to 675 days the tumours with statistically significantly increased incidences were observed in liver - 41% in males and 75% in females, in forestomach - 41% in males and 31% in females, and in lungs - 69% in males and 42 % in females (*Ohgaki et al, 1984*). It has to be noted, however, that the tumours in IQ-treated mice were first observed from day 504 and on of experimental feeding, thus after much longer exposure than in the present study.

In contrast, the tumour incidence of C57BL/6ByA and Eμ-*pim*-1 transgenic mice exposed to 0.03% IQ in the diet throughout 24 weeks and observed for total of 40 weeks was sporadic and not statistically significantly increased compared to the controls (*Sørensen et al, 1996*). The recorded tumours in IQ-treated transgenic and wild type mice were of spontaneous background pathology and were located in the haemolymphoreticular system (four Eμ-*pim*-1 transgenic females, one C57BL/6ByA female) and in the liver (one Eμ-*pim*-1 transgenic female, two C57BL/6ByA females), in lungs (one C57BL/6ByA female), in forestomach (one Eμ-*pim*-1 transgenic male) while all IQ-dosed C57BL/6ByA males were tumour negative. The negative tumour results with IQ in C57BL/6ByA mice were ascribed to too short experimental period of that short-term carcinogenicity testing. The negative results with IQ in Eμ-*pim*-1 transgenic mice supported that the carcinogen, which does not have a lymphoid system as a target was not recognized in this model.

In the present study, the first tumour of IQ-dosed mice was recorded in the liver on day 292 of the

experiment in the HMGCR/mts1 tg579 TR male while other tumours were first observed at the termination, when the age of mice was 469 days. Thus, it can be speculated, based on the previous (*Sørensen et al, 1996*) and present results, if our negative (from the statistical point of view) findings on carcinogenicity of IQ in the C57BL/6A and HMGCR/mts1 tg579 TR mouse strains may indicate that a relatively short exposure to IQ (24 or 26 weeks) does not lead to development of tumours later in life. However, it is probable that the negative results in the present study were due to too short exposure (too low cumulative intake) and/or due to too short post treatment observation period keeping in mind that tumours in CDF_1 mice (*Ohgaki et al, 1984*), rats (*Takayama et al, 1984*) and in nonhuman primates (*Adamson et al, 1994*) were recorded after a longer exposure. Furthermore, a possible strain difference in susceptibility to IQ and the use of different diets should be kept in mind as factors of importance when considering the reasons for our negative findings and a positive carcinogenic response to IQ in mice reported by Ohgaki et al. (1984).

Previous studies demonstrated enhanced levels of S100A4(mts1) protein in the blood of aged (more than 16 month-old) HMGCR/mts1 tg579 TR mice (*Ambartsumian et al, 2001*). The enhanced levels correlated with development of hyperproliferative disorders, such as hemangioma in the liver and stenosis in the lung. It was proposed that tissue-specific down-regulation of expression of S100A4(mts1) was altered in the aged transgenic animals. Upon aging the strictness of repression was deregulated in some of the animals resulting in accumulation of S100A4(mts1) in the tissues with subsequent development of pathological changes (*Ambartsumian et al, 2001*). Therefore it could be expected that treatment with carcinogens would enhance both the frequency and the onset of pathological conditions described in the HMGCR/mts1 tg579 TR animals. In the present study, however, the serum levels of S100A4(mts1) protein in the control and IQ treated HMGCR/mts1 tg579 TR mice were not significantly different from those in C57BL/6ByA mice. While the lack of increase of Mts1(S100A4) serum levels of the untreated HMGCR/mts1 tg579 TR mice could be explain by their less advanced age in the present study compared to the previous one (*Ambartsumian et al, 2001*), the lack of difference between the untreated and treated HMGCR/mts1 tg579 TR mice of both sexes indicates no effect of the applied IQ-treatment on this parameter. It is also in accordance with single incidences of the proliferative lesions and the recorded (low) tumour incidence in the treated transgenic mice of both sexes compared to the controls.

Two of the tumours diagnosed in the IQ-treated HMGCR/mts1 tg579 TR males and one in a C57BL/6ByA female represented rare types (Table 4). This included a liver hemangiosarcoma characteristic for the old mice with an incidence in C57/Bl/6 strain below 3% for animals older than 24 months (*Turusov & Mohr, 1994*) and colon adenocarcinoma with the reported incidence of one tumour in the population of 914 C57/Bl/6 mice at 18 months of age (*Rowlatt et al, 1969*). The recorded rare types of tumours in the IQ-treated HMGCR/mts1 tg579 TR males together with the recorded pre-neoplastic lesions in the liver (which could develop into neoplasia if the study was conducted for a longer period of time) in both sexes could serve as an indicator of a carcinogenic effect of the compound in the transgenic mice according to the criteria used by the International Life Sciences Institute's Alternatives to Carcinogenicity Testing Program (*Mortensen et al, 2002*). The present study was conducted only for a limited time period. Since the pathological changes in the HMGCR/mts1 tg579 TR mice were earlier observed in older animals (*Ambartsumian et al, 2001*), it can be suggested that the changes observed in the HMGCR/mts1 tg579 TR males treated with IQ could become much more pronounced with time.

In conclusion, a limited bioassay in C57BL/6BYA

and HMGCR/mts1 tg579 TR mice lasting for a total of 53 weeks during which the mice were the exposed to 0.03% of the fried food mutagen IQ in the diet for 26 weeks did not demonstrate a statistically significant carcinogenic response to the compound in both genotypes. The results in HMGCR/mts1 tg579 TR mice obtained under current experimental conditions suggest that 53 weeks may be not a sufficient time span to demonstrate a carcinogenic potential of a test compound in this mouse model.

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