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Long term tumorigenicity of a single application of indomethacin or Amuno® in adolescent and in adult male Sprague Dawley rats

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With 3 figures and 5 tables

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Summary

200 adolescent (Group I) and 200 adult male Sprague Dawley rats (Group II) were divided into 4 subgroups of 50 animals each. Animals were treated on the 29th (Group I) or 98th day of life (Group II) either with acetone or Amuno® carrier in acetone or Amuno® on acetone (2.5 mg indomethacin/100 g animal weight in acetone) or with the pure substance indomethacin 2.5 mg/100 g of animal weight in acetone, giving a single application on the shaved dorsal skin. Subsequently the animals remained under observation until their deaths, followed by autopsy and histopathologic examination of several organs. The rats treated with Amuno® of indomethacin in the adolescent stage showed lower body weights and a shorter total survival time. The adolescent animals treated with Amuno® or indomethacin showed a significantly higher rate of interstitial testicular tumors of the Leydig tumor type, adenomas and adenocarcinomas of the small and large intestine as well as hepatocellular tumors. The total number of neoplasias was higher in the animals treated with Amuno® or indomethacin compared to those treated with acetone or carrier with acetone. Application of Amuno® or indomethacin also resulted in a higher rate of hyperplasias (sole of foot, lymph nodes, prostate). The impairment of the synthesis of prostaglandins caused by indomethacin apparently results in starting complex pathomechanisms which have effects until the late death of the animals. Adolescent animals were affected more frequently by the application of indomethacin as were already adult animals.

Introduction

In previous experiments on two stage carcinogenesis we had investigated the role of various substances which interfere either with the initiation of the promotion step (19). Since tumorigenesis in some human organs is enhanced by inflammatory processes we were interested in investigating the influence of an inhibitor of inflammation in the framework of a two stage carcinogenesis protocol.

Prior to setting up such an experiment we investigated in a pilot study the effect of a single application of the antiinflammatory, therapeutically used drug Amuno® (active substance indomethacin) in acetone to the dorsal skin of 50 adolescent male rats. Concomitant with a control group which received only a topical application of acetone the animals were observed until their natural death.

To our surprise we noticed in the Amuno® treated animals an unexpectedly high incidence of testicular interstitial cell tumors of the Leydig typ, which we had never observed in any other experiment. The therapeutically used Amuno® contains not only the active substance indomethacin but also a carrier substance. Therefore our pilot experiment did not provide reliable information on the effect of indomethacin alone. The pilot study was also carried out in adolescent male animals and it was mandatory to investigate the effect of the substances also in adult male rats. In the present study we investigated the presumptive tumorigenic effects of a single topical dose of Amuno®, Amuno® carrier substance, and pure indomethacin in a lifelong experiment with both adolescent and adult male rats. Male rats were used exclusively due to the wellknown tendency of female rats to die prematurely from spontaneous mammary carcinomas. Distinct tumorigenic effects were observed in the adolescent, not in the adult animals.

Material and methods

1. Animals, experimental setup

Twohundred 21 days old and twohundred 90 days old male Sprague Dawley rats (Süddeutsche Versuchstierfarm, Tutt-

lingen) were used in the experiment. The animals were housed in individual cages (Macrolon type III) under an artificial day/night rhythm and received Altromin Standard diet 1334 and water ad libitum.

21 days old male rats are still juvenile whereas 90 days old animals are fully matured. With regard to the expected testicular tumors the use of both mature and immature animals seemed important to us.

At the beginning of the experiment the adolescent animals were 28 days old (Group I) and weighted 76–84 g, the adult animals were 97 days old (Group II) and weighted 328–341 g. Animals of Group I and II were randomized under 4 subgroups of 50 animals each and marked at the ears.

Control animals (I, 1 and II, 1) received a single topical application of 1 ml of acetone per 100 g body weight in the back skin which shaved 24 h to application of the solvent.

Indomethazin is applied cutaneously for therapy and hereby becomes fully effective. That is why we could expect a rapid penetration of the drug after cutaneous application.

Subgroups I. 2 and II. 2 received 21.3 mg Amuno® carrier substance per 100 g body weight dissolved in acetone.

Animals of subgroups I. 3 and II. 3 received 2.5 mg of indomethacin per 100 g of body weight, dissolved in acetone.

Animals of subgroups I. 4 received Amuno® in concentration of 2.5 mg indomethacin + 21.3 mg carrier substance (reg. no A678 G8658, no. 95740) per 100 g body weight, dissolved in acetone. (For the different experimental groups see also Table 1).

Table 1. Schedule of application; substances and their attachement of experimental groups. Local application to the shaved back skin on day of life 29 (adolescent animals, weight 76–84 g) resp. on day of life 98 (adult animals, weight 328–341 g).

Substance/ 100 g body weight	Local Application	
	Group I Adolescent male animals/subgroup n = 50	Group II Adult male animals/subgroup n = 50
Acetone 1 ml	I/1	II/1
Carrier 21.3 mg	I/2	II/2
Indomethacin/ 2.5 mg-Acetone 1 ml (Charge Sp 1599 Lot. Nr. ITA-095	I/3	II/3
Amuno® 2,5 mg Indomethacin + 21.3 mg Carrier + Acetone 1 ml (Reg. Nr. A678 G658, Nr. 95740	I/4	II/4

All animals were observed until their natural death. From every animal 25 organs or parts of organs were removed routinely together with any other tissue showing pathological changes. The material was fixed in neutral formalin and then paraffinized. Histological sections were stained with hematoxylin/eosine (HE). The histopathological examination was done by the first author of this study (KG). All findings were recorded on the histopathological record sheets, entered into the German Cancer Center Research Computer, and evaluated in-house at the Department of Biostatistics.

Statistical Analysis

- Data Acquisition and Retrieval

Based on the comprehensive histopathology of each animal documented on special record sheets developed at the Institute of Experimental Pathology at our Center, computer programs were developed at the Department of Biostatistics at the DKFZ for data acquisition and retrieval taking into account location and tissue of histological findings; classification into malignant and benign tumors, hyperplasia, metastases, and other alterations. Up to ten specific histological characterizations were considered.

Histological information was coded automatically by a four digit number which could be assigned to each of the 200 individual animals as often as a histological finding had to be documented. In doing so we were able to print out each animals histological description as well as to search for selected combinations of histological characterizations.

Survival times of the animals were recorded separately be the program package ADAM (41) together with the animals identification, Group I or II adherence, and treatment. Furthermore these data were matched to the individual histological data of each animal.

The data analysis was stratified according to adolescent animals (Group I. 1–4) and adult treated animals (Group II. 1–4).

- Statistical analysis

Statistical analysis of the experiment was performed according to the recommendation of PETO et al. (34) and GART et al. (18). Based on the computerized histological documentation of malignant and benign tumors, hyperplasias, metastases, and other alterations were calculated overall as well as site and tissue specific frequencies for the two groups of adolescent (I) and adult (II) animals and the four subgroups each ("Control" = I. 1 resp. II. 1; "carrier" = I. 2 resp. II. 2; "indomethacin" = I. 3 resp. II. 3; "Amuno® = I. 4 resp. II. 4) by specially designed computer programs. Frequencies of the control and the three treatment subgroups as well as of the two combinations Control/carrier and indomethacin/Amuno® were compared by Fisher's exact test or by the Chi-square test (15). For combination over different strata we used the Mantel-Haenszel procedure.

Mortality was analyzed by methods for failure time data (23). For the comparison of the survival times in the respective groups we used the Wilcoxon Rank Sum test, since no censored data were present, and we calculated the median with 95% confidence intervals. Methods for censored survival times were applied for the assessment of the time to death for malignant or benign tumors (9, 10).

Prevalence of malignant tumors observed at death were analyzed by the method proposed by PETO et al. (34) as it has been implemented by ROSENKRANZ (36). The adaptive interval selection method of PETO et al. (34) was used to determine the time subintervals for which prevalences were calculated, see also GART et al. (18). Prevalences were reported as the ratio of the number of malignant tumors and the number of animals at risk in that interval for each treatment group. In addition, total cumulative prevalence was calculated as the total number of malignant tumor deaths divided by the total number of animals per group. Repeated measurements of the animals' weights and

the consumption of water and food were analyzed by graphical tools and by the KRUSKAL-WALLIS test (22).

All calculations were performed by software developed at the department of Biostatistics, especially the programm package ADAM (41) and CAGEN (36).

Results

Survival times

Figures 1 and 2 show the mortality curves of the 4 subgroups of main group I (Fig. 1) and main group II (Fig. 2) from the date of application of the various substances until death. Whereas no statistically significant differences are seen in the 4 subgroups of the adult animals (Fig. 2), subgroup I. 4 (Amuno®) of the adolescent animals differs markedly from the remainder of the subgroups between days 500 to 700 after application of Amuno® (Fig. 1). Asterisks indicate animals in which adenocarcinomas of the intestinal tract could be identified during autopsy.

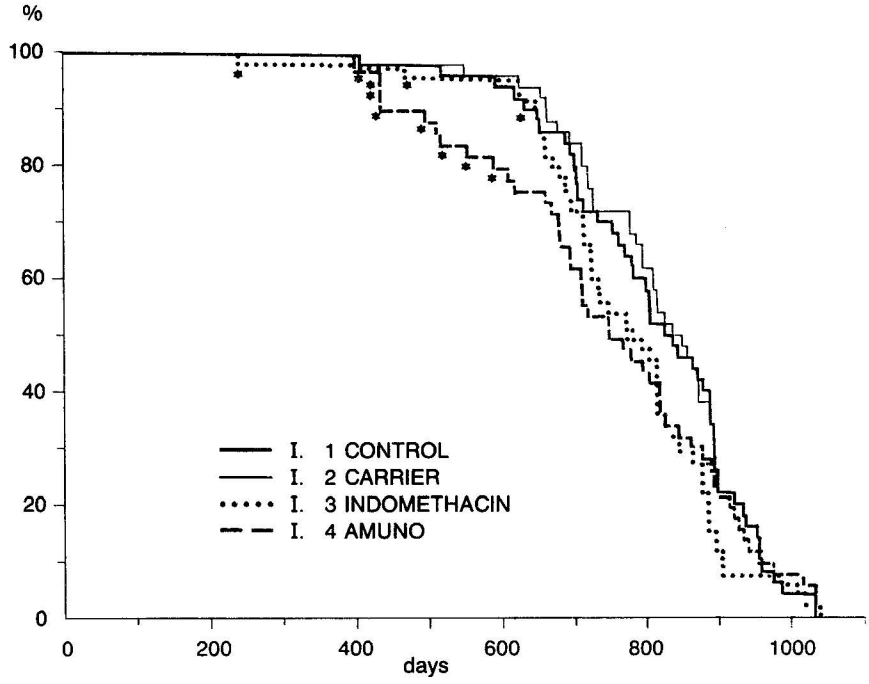

Fig. 1. Mortality and survival curves of the adolescent male animals in main group I, at start of experiment 28 days old, 76–84 g. * death of an animal with intestinal carcinoma.

Table 2 contains a comparison of the average survival times. The difference between the life expectancy of groups I. 3 and I. 4 and the life expectancy of the control group was statistically significant ($p = 0.07$ and 0.04, respectively). The differences in the subgroups II. 1 to II. 4 was in no case significant. Death with a malignant tumor was earlier in the indometacin and in the Amuno® group (Median 882 resp. 849 days) compared with the control and the carrier group (Median 960 resp., 933 days) among the young treated rats (main group I). These differences were statistically significant ($p = 0.0009$ with the log rank test). Compared to the control group (I. 1), carrier (I. 2), indomethacin (I. 3) and Amuno® (I. 4), treated animals had a relative risk of dying with a malignant tumor of 1.4, 2.5, and 2.4 respectively. No differences were observed in Group II, neither with respect to death with a benign tumor.

These results could be confirmed by evaluating the prevalence of malignant tumors at the animals' death (Tab. 3). There were again statistically significant differences between the four groups with a relative risk increasing from 1.4 in the carrier group to 1.9 and 2.1 in the indomethacin and in the Amuno® group in the young treated animals only. Prevalence analysis of tumors according to location (colon, mammary gland, skin, testis) confirmed the overall results obtained by the evaluation of the frequency numbers in table 3.

The autopsy protocols and the histological findings also include non-tumorigenic diseases of the lung (pneumonia), the heart (myocarditis), the hepatic and bile ducts (choloangitis), as well as inflammation of kidney (nephritis, pyelitis), and of the urinary tract (ureteritis, urocystitis).

Body weights during experiment

From week 3 onwards, the groups of the adolescent animals treated with indomethacin and AmunoR (I. 3 and I. 4, respectively) possessed a lower average body weight compared to the two control groups (I. 1 and I. 2, respectively). This is shown in figure 3. This figure compares the weight development of the control group with the group of the animals treated with Amuno® with regard to the adolescent animals (I. 1/I. 4) as well as to the adult animals up upon start of the experiment. Compared with the controls, the adolescent animals treated with Amuno® were behind, and this could be ascertained statistically up to week 107 at 5% (Kruskal-Wallis test with subsequent multiple comparisons according to Dunn; see also HOLLANDER and WOLFE

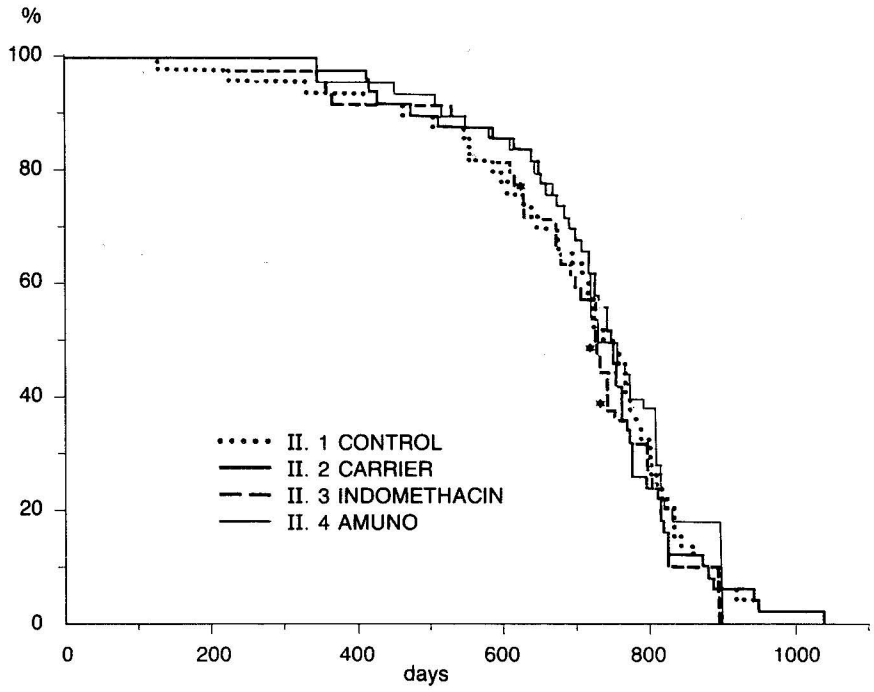

Fig. 2. Mortality and survival curves of the adult male animals in main group II, at start of experiment 97 days old, 328–341 g. * death of an animal with intestinal carcinoma.

Table 2. Median survival in the four subgroups of adolescent (I) and adult (II) male Sprague Dawley rats with 95% confidence limits. Each subgroup (1–4) comprised a total of 50 animals. Survival time was measured from start of treatment (day 29 for adolescent and day 98 for adult animals, respectively) until natural death or sacrifice because of being moribund. Statistical tests for differences between the three subgroups (2, 3 and 4) and the control (1) were performed by the one-sided WILCOXON rank sum test. Median time to death with a malignant tumor are given in parentheses.

Subgroup	Adolescent animals (I)$^{+}$		Adult animals (II)$^{++}$	
1. (Control)	834.5 773–891	(960)	750 699–790	(840)
2. (Carrier)	847 798–890 p = 0.6	(933)	751 720–772 p = 0.5	(828)
3. (Indomethacin)	788.5 728–829 p = 0.07	(882)	730 680–772 p = 0.3	(803)
4. (Amuno®)	760.5 695–828 p = 0.04	(849)	741 717–808 p = 0.7	(893)

$^{+}$ survival time beginning with day 29 of life; $^{++}$ survival time beginning with day 98 of life

Table 3. Prevalence of malignant tumors at death in adolescent treated (Group I) and adult treated (Group II) male Sprague Dawley rats in time intervals determined according to the adaptive interval selection method of PETO et al. (34). Reported are the numbers of animals dying with a malignant tumor among all deaths in the respective time interval. Percentages are given in parentheses.

Survival time subintervals	Group			
	Control	Carrier	Indo-methacin	Amuno®
I				
0– 654	2/7 (29)	0/4 (0)	4/7 (57)	3/12 (25)
655– 723	1/7 (14)	3/9 (33)	5/10 (50)	5/11 (45)
724–1100	10/36 (28)	15/38 (40)	16/33 (48)	18/27 (67)
Total cumulative prevalence	13/50 (26)	18/50 (36)	25/50 (50)	26/50 (52)
II				
0– 466	1/5 (20)	2/4 (50)	2/4 (50)	0/3 (0)
467– 890	19/41 (46)	16/42 (38)	20/41 (49)	16/38 (42)
891–1100	1/3 (33)	0/2 (0)	3/5 (60)	5/9 (56)
Total cumulative prevalence	21/50 (42)	18/50 (36)	25/50 (50)	21/50 (42)

(22)). From week 107 onward, these differences were still existing in tendency, however, not statistically significant due to the increasing loss of animals. Such differences were not detected in adult animals. In order to avoid a high multiplicity of hypothesis testing and to assure highest statistical power at the same time the results were only reported for the significance tests between the combination control + carrier (= 100) and the combination Indomethacin + Ammuno (= 100). But, the frequencies of each single treatment group were additionally reported in Table 4. No further statistically adjustments for multiple comparisons were made.

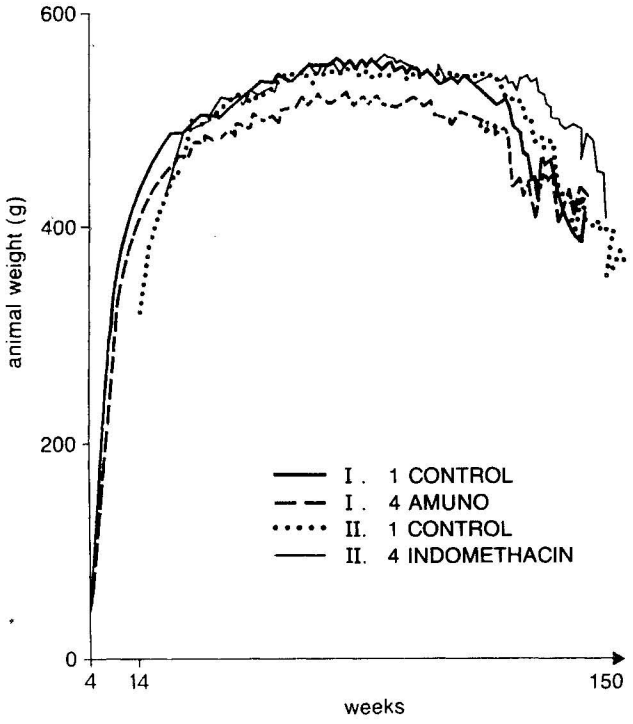

Fig. 3. Comparison of average body weight of control group and Amuno® in adolescent animals (I. 1–I. 4) and adult animals (II. 1–II. 4).

Tumor incidence and tumor spectrum

82 of the 200 animals in Group I tested as adolescent rats had developed malignant tumors at final autopsy, 13 (= 26%) and 18 (= 36%) of them being in the control and carrier groups and 25 (= 50%) and 26 (= 52%) in the indomethacin and Amuno® groups. The difference between 31% and 51% in the two combinations is statistically significant ($p = 0.0004$). When we compared the indomethacin and the Amuno® group separately with the controls in Group I both had significantly more malignant tumors ($p < 0.02$). In contrast to this, 87 of the tumor bearing rats of the group of adult rats (Group II) were equally distributed over the two combinations (22 and 19 in the control/carrier combination and 25 and 21 in the indomethacin/Amuno® combination; $p = (0.5)$.

Table 4 shows the incidence of tumors of the 11 most frequently affected organs. Additional neoplasias in other organs or tissues are not shown in the table due to their low number, with regard to intestinal tumors (adenocarcinoma, adenoma) there existed a significant difference between the adolescent animals of the control groups and the Amuno® or indomethacin treated animals.

A survey of the tumors listed in table 4 according to the histological type and classification did not bring about additional information. For completion, this histological types observed in the organs are summarized as follows:

Intestine
benign: Several histiocytomas
malignant: Adenocarcinomas, (more frequently in the small intestine than in the large intestine, mainly singular; in 2 cases-Groups I, 3+4-with lymphonodular metastases);
some leucoses were observed, they are not included in the numbers of table 4

Liver
benign: Hepatocellular adenomas
malignant: Hepatocellular carcinomas
The tumors which metastasized into the liver from other organs are not included in the numbers of table 4

Testes
benign: Interstitial cell tumors of the LEYDIG-Type
malignant: Interstitial cell tumors of the LEYDIG-Type

Pancreas
benign: Islet cell adenomas, scarcely some adenomas of the exocrine gland.
malignant: Islet cell carcinomas

Skin
benign: Fibroepithelioma, fibromas, sebaceous adenomas
malignant: squamous cell carcinomas, basaliomas

Glandular Stomach
malignant: Adenocarcinomas

Mammary gland
benign: Fibroadenomas
malignant: Adenocarcinomas

Thyroid gland
benign: Follicular adenomas
malignant: Follicular carcinomas

Adrenal gland (medulla)
benign: Pheochromocytomas
malignant: Pheochromoblastomas

Pituitary gland
benign: stem cell adenomas
malignant: stem cell carcinomas

Benign tumors occurred very often in Groups I and II, and the numbers were almost equal in each of the four subgroups (Control I. 1; II. 1 = 41 and 42; Carrier I. 2; II. 2 = 43 and 43; indomethacin I. 3; II. 3 = 42 and 41; Amuno® II. 4; II. 4 = 40 and 43). Benign brain tumors were observed in the carrier and in the Amuno® subgroups only (both n = 2).

Liver neoplasms were more frequent as well as interstitial testicular tumors of the Leydig tumor type in the indomethacin/Amuno® subgroups. This tumor type was discriminated from Leydig cell hyperplasia by its local autonomous growth, whereas the latter showed a merely diffuse segregation. The Leydig cell tumors were benign with the exception of one malignant metastasizing interstitial cell carcinoma. These benign tumors occurred significantly more often in adolescent and adult animals ($p = 0.005$ and 0.03 respectively) compared to the control animals. Statistical differences from those organs which frequently showed tumors in the controls (pancreas with islet cell tumors, thyroid gland with follicular adenomas, pituitary gland with stem-cell adenomas) could not be ascertained.

Table 4. Frequency of malignant and benign tumors observed in adolescent (Group I) and adult (Group II) male Sprague Dawley rats at 11 main organs. Numbers (malignant/benign) are given for each of the four subgroups (1−4) as well as for the combination Control/Carrier (1 + 2 = 100) and for the combination of the Indomethacin/Amuno® subgroup (3 + 4 = 100). Statistical test of differences between combination subgroups 1 + 2 and 3 + 4 were performed by FISHERS exact test both for malignant and benign for the organs with footnotes.

Organ*		Control (n = 50)	Carrier (n = 50)	Control + Carrier (n = 100)	Indomethacin (n = 50)	Amuno® (n = 50)	Indomethacin + Amuno® (n = 100)	Total (n = 200)
Intestine	I	0/0	0/0	0/0	3/1	8/2	11[b]/3	11/3
	II	0/0	0/0	0/0	1/0	1/0	2/0	2/0
Liver	I	0/0	1/0	1/0	1/4	2/3	3/7[c]	4/7
	II	0/1	0/2	0/3	1/0	0/2	1/2	1/4
Testes	I	0/1	0/0	0/1	0/6	1/5	1/11[d]	1/12
	II	0/0	0/0	0/0	0/1	0/5	0/6[d]	0/6
Pancreas	I	5/10	2/21	7/31	1/15	2/8	3/23[a]	10/54
	II	3/13	2/18	5/31	0/17	2/18	2/35	7/66
Skin	I	1/4	3/3	4/7	4/5	3/7	7/12[a]	11/19
	II	3/5	2/8	5/13	3/9	6/3	9/12	14/25
Glandular stomach	I	0/0	0/0	0/0	1/0	0/0	1[a]/0	1/0
	II	0/1	2/0	2/1	1/0	1/0	2/0	2/0
Mammary gland	I	2/2	2/1	4/3	2/5	1/2	3/7	7/10
	II	0/6	0/4	0/10	5/3	0/2	5/5	5/15
Thyroid gland	I	0/22	0/22	0/44	2/26	3/22	5/48	5/92
	II	4/25	1/26	5/51	2/30	0/30	2/60	7/111
Adrenal gland (med)	I	2/12	5/17	7/29	5/10	4/12	9/22	16/51
	II	7/10	8/11	15/21	6/13	8/18	14/31	29/52
Pituitary gland	I	1/27	0/27	1/54	1/29	3/22	4/51	5/105
	II	1/26	0/32	1/58	1/24	2/24	3/48	4/106

Comparisons with the combination Control/Carrier:
a) no statistically significant difference ($p > 0.05$)
b) statistically significant difference for malignant tumors in I only ($p = 0.007$)
c) statistically significant difference for benign tumors in I only ($p = 0.014$)
d) statistically difference for benign tumors in I and II ($p = 0.005$ and $p = 0.03$)
* For further classification see text.

Tumors of the skin were mainly located in the shaved back skin i.e. the area of the application of the test substances. A statistically difference between the combination group of the animals treated with indomethacin/Amuno® and the collective control/carrier groups could not be detected.

1−2 carcinomas of the mammary glands appeared in all four subgroups I.1−I.4, but only the indomethacin group II. 3 (n = 5) being borderline significant compared with the control II. 1 (n = 0; p = 0.056).

The tumors of the pancreas were mainly islet cell tumors, and the tumors of the adrenal gland mainly pheochromoblastomas and pheochromocytomas. In case of the tumors of the pituitary gland these were adenomas of the stem cell-type.

Hyperplastic lesions; other histopathologic findings

Table 5 contains information about individual non-neoplastic lesions in organs or parts of the body. Differences in the patho-anatomic findings between groups were not significant with regard to the urinary tract. A striking fact,however, was a hyperplasia of the epidermis frequently combined with inflammation which occurred in the skin of the foot sole. This could already be detected in living animals. In addition to this finding, rigidity of limbs occurred in these animals, which showed up when the extremities had to be extended on the autopsy table. The histopathologic examination of knee-joint synovia, cartilage and bone revealed no differences between the groups; however, comparatively frequent degenerative defects in cartilage could be observed.

Not included in Table 5 were single findings in the liver. Cholangiosis as well as nodular hyperplasias were found singularly and equally distributed in the control groups as well as in the experimental groups.

In the prostate, adenomateous hyperplasias could be demonstrated in group I with a significant frequency. Within these groups phenomena of lymphonodular hyperplasias were also more common. In contrast, hyperplasias in the anterior lobe of the pituitary gland and in the adrenal medulla were equally distributed among the experimental groups.

Table 5. Frequency of hyperplastic lesions observed in adolescent (Group I) and adult (Group II) male Sprague Dawley rats observed in 7 organs or tissues. For explanation see Table 3.

Organ tissue		Control (n = 50)	Carrier (n = 50)	Control + Carrier (n = 100)	Indomethacin (n = 50)	Amuno® (n = 50)	Indomethacin + Amuno® (n = 100)	Total (n = 200)
Skin	I	3	4	7	10	17	27[b)]	34
(Foot sole)	II	7	18	25	18	10	28	53
Prostate	I	6	11	17	11	19	30[c)]	47
gland	II	8	12	20	11	10	21	41
Lymph	I	0	1	1	10	3	13[d)]	14
nodes	II	0	4	4	7	2	9	13
Urinary	I	12	9	21	12	4	16[a)]	37
tract	II	3	9	12	5	10	15	27
Kidney	I	4	0	4	0	0	0	4
(pelvis)	II	3	2	5	1	1	2	7
Pituitary	I	13	12	25	11	11	22	47
gland	II	13	8	21	17	14	31	52
Adrenal gland	I	17	12	25	11	11	22	47
(med)	II	12	11	23	15	12	27	52

Comparisons with the combination Control + Carrier:
a) no statistically significant difference ($p > 0.05$)
b) statistically significant difference in I only ($p = 0.00025$)
c) statistically significant difference in I only ($p = 0.03$)
d) statistically significant difference in I only ($p = 0.001$)

Discussion

The present study was motivated by unexpected findings in a previous pilot experiment. When we observed 400 male Sprague Dawlex rats during total life we could prove that one single local application of indomethacine or Amuno® was sufficient to induce general as well as local damage, including neoplastic and hyperplastic lesions. Individual lesions showed significant differences when compared with the controls. These lesions were intestinal tumors, testicular tumors of the Leydig cell type, epidermal hyperplasias of the foot sole and lymph node hyperplasias. With regard to other lesions, only tendencies could be detected.

A comparison of our tumor incidences found in this prospectively designed and randomized control experiment, refers to a total of 200 control animals of the Groups I, 1 + 2 resp. II, 1 + 2, versus 200 treated animals of the Groups I, 3 + 4 resp. II, 3 + 4. A comparison with datas of spontaneous tumors in rats of the literature (3, 26) is less informative. The animal material offered from the breeder changes continuously. Formerly we had reported on spontaneous tumors in 100 male and 100 female controlled Sprague Dawle rats until death (26). BODE et al. (3) described tumors in 924 rats without subdivision into males and females. In both publications tumors of the skin, digestive apparatus, islet cell tumors, thyroid gland, pituitary and adrenal glands developed spontaneously. Compared to our 200 control animals, the amount of tumors in the digestive apparatus was almost equal. In contrast, spontaneous tumors arose more frequently in this experiment in islet cells, in the thyroid gland, pituitary and adrenal glands.

Our experiment provided patho-anatomic findings and a further basis to discuss the complex spectrum of indomethacin which we already mentioned in the introduction. The anti-tumorigenic, even chemoprophylactic effect deduced from the anti-inflammatory property (27, 28) had been described before by several teams with regard to experimentally induced autochthonous tumors (5, 8, 27, 29, 30, 35, 37). Inhibitory effects on growth and metastatic spread of transplanted tumors were also determined (mammary gland: 17, 24, 25; colon: 31, 39; prostate: 8). On the other hand, absence of anti-neoplastic action was observed. Thus, DMBA-induced cancerogenesis in the cheek pouch of the Syrian hamster was not affected (16, 20).
The tumor-inhibitory effect on the development of DMBA-induced breast cancer (see above) described by other authors could also not be confirmed (1, 27, 33). Apparently, dietetic factors, including the share of nutritive fat, exert an additional influence. Inhibitory effects on the development of dimethylhydrazin-induced colon adenocarcinoma could also not be observed (4, 32). This also applies to induced carcinoma after administration of urea derivatives or of methylazoxymethanol. The alveolar cell carcinoma of the mouse induced by benzo(a)pyrene was not affected (2). DANZI (6) even described a promoter effect of the indomethacin on the dimethylhydrazin-induced adenocarcinoma of the colon.

We cannot comment on the complex biochemical effects of indomethacin on the basis of our patho-anatomic data. But the blocking of prostaglandin synthesis apparently constitutes the center of the action, presumably via the cyclooxygenase pathway of the arachidonic acid metabolism (7). According to RUI (38), indomethacin prevents prolactin-

induced effects. These also include desensitization of the prostaglandin-E 1-dependent adenyl cyclase. Prostaglandin could also induce similar changes in the prostaglandin-E 1-dependent cyclase of rat Leydig cells in vivo and in other prolactin-dependent target organs. Thus, our findings on the occurrence of interstitial cell tumors of the Leydig type gain additional importance. GROTJAN et al. (21) and ERICHSEN et al. (11, 12, 13, 14) dealt with the biochemistry of cultivated and transplanted cells of a Leydig cell tumor under the influence of indomethacin. The hyperplasis observed by us in the prostate, i.e. the typical target organ of prolactin action, also point to hormonal effects.

We could show that one single application of indomethacin or Amuno® had general as well as systemic consequences. The latter could even be determied at the end of the experiment fixed for total life span. The dose administered was the dose recommended on the package circular of Amuno® available on the market for longterm medication in man (daily doses between 50 and 200 mg of indomethacin). THUILLIER et al. (40) indicate for indomethacin 50 mg/kg of body weight for oral LD_{50}-dose with regard ro rats. This oral dose corresponds to the double amount of the substance administered by us on the shaved back skin by single local application. Thus our dose was not excessive. If affected in particular male adolescent rats whose body weights lagged behind the body weight of the controls during the whole lifetime. Total lifetime was also reduced. Body weight as well as total lifetime are complex factors. Their pathogenesis cannot be clarified neither by our observation during the whole life nor by the histopathologically examined tissue samples collected during autopsy. The reduction in weight can be assessed most likely as sign of intestinal resorption defect, particularly since the system of intestinal surface epithelial in small and large intestine is considered to be a target organ of indomethacin action.

Indomethacin preferentially affects young animals. Only tests carried out with these groups compared to groups with adult rats at begin of the experiment revealed the extent of the defects.

Thus, the findings of our experiment are of paradigmatic importance for general tests on substances within the framework of pharmaceutical testing. These tests are being carried out e.g. within the framework of drug testing using adult animals. The effects determined by us on body weights and total lifetime could not have been observed in adult animals.

Our experimental setup is unsuitable to further clarify the complex and often hormone-controlled biochemical series of reaction with regard to the effect of indomethacin or Amuno® in their pathogenesis. Having shown an increased suspectibility of the adolescent compared to the adult organism, the question arises whether the prenatal organism is more sensitive to indomethacin (or Amuno®). The application of these substances in pregnant women seems at least problematic, if not inadvisable.

Complex modes of action are apparently responsible for the fact that we could identify the interstitial testicular cells, the rapidly proliferating glandular epithelia of small and large intestine as well as hepatocytes of the liver as special target organs of the indomethacin (Amuno®) action. The result was a higher tumor yield in the target organs mentioned. Furthermore, an increase in the total number of all neoplasias observed was to be noted as well as in the number of tumors classified histopathologically as malignant.

On the other hand, the islet cells of the pancreas, basal stem cells of the anterior lobe of the pituitary gland and the phaeochromocytes of the adrenal medulla can be ruled out as target organs since in these organs the number of tumors observed in all subgroups remained the same. In total, adolescent animals were also more sensitive in this case. Apparently, the ability for proliferative activity plays a role, i.e. the ability to increased reaction on proliferation stimuli in the sensitive systems. This hypothesis is also confirmed by the observation that hyperplaseogenous defects were more frequently observed in animals treated with indomethacin or Amuno®.

In summary it can be stated that the tumorigenic effect of indomethacin or Amuno® on the interstitial testicular cells of mainly male adolescent Sprague Dawley rats as well as the additional tumorigenic effects on interstinal glandular epithelia and on hepatocytes only covers a small part of the spectrum of indomethacin (Amuno®). We were able to affect complex metabolic processes by one single local application and were able to reveal side effects unknown up to this point. In order to achieve this, standardized animal experiment usually employed as standard tests on drugs would not have been suitable. It was only by the lifetime experiment using young animals as well as adult animals at the time of the experiment that we were able to detect substance-related effects which seem to trigger proliferative abilities in the organism as a whole as well as in individual organs. According to the findings of our experiment we are of the opinion that the prenatal organism is more in danger than the postnatal adolescent organism and the warining is founded not to administer indomethacin or Amuno® in pregnant women or adolescents.

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