DOSE–ONCOGENIC EFFECT RELATIONSHIPS FOR N-NITROSODIMETHYLAMINE ON *Rana temporaria* – A NEW EXPERIMENTAL ANIMAL IN CANCER RESEARCH

V. V. Khudolei

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The writer showed previously that the frog *Rana temporaria* is highly susceptible to the carcinogenic action of nitroso compounds [1].

The object of this investigation was to study the relationship between the oncogenic effect of N-nitrosodimethylamine (NDMA) and its dose. Establishment of this relationship is essential in order to characterize this experimental animal as a new model in cancer research.

EXPERIMENTAL METHOD

Experiments were carried out on 285 frogs (*Rana temporaria*) of both sexes aged 1-1.5 years. The animals were kept in sterile containers (five frogs in each container, 0.5 liter water per frog) at 6-10°C. The water was changed daily with addition of NDMA in various concentrations, equivalent to 1/30-2/3 $LD_{50/30}$ established in toxicity experiments [2]. In group one, 65 frogs were treated with NDMA in a dose of 1 ppm. In group two 110 animals received NDMA in a dose of 5 ppm. NDMA was used in concentrations of 10 and 20 ppm in groups three (62 frogs) and four (48 frogs). The animals were exposed to the carcinogen for the rest of their life, which amounted, under these experimental conditions, to 30 weeks for the frogs of group one, 29 weeks for group two, 16 weeks for group three, and 11 weeks for the frogs of group four. The control consisted of 270 intact frogs on which observations were made for 56 weeks. The liver, kidneys, and spleen of all animals dying in the course of the experiments from the toxic action of NDMA or from tumors were subjected to morphological analysis. For statistical analysis of the data the chi-square method (frequency of tumors) and t-test (mean latent period) were used. The results are shown in Table 1.

EXPERIMENTAL RESULTS

The toxic action of NDMA was exhibited 2-3 weeks after the beginning of the experiment and expressed as a reduction in motor activity of the animals. Foci of necrosis were observed in the liver and kidneys at this time, most often in the frogs of groups three and four. Diffuse and focal hyperplasia of the liver tissue, disorganization of the trabeculae, and the formation of polymorphic cell complexes were found after 4-6 weeks. Proliferation of the epithelium of the bile ducts and hyperplasia of the epithelium of the renal tubules were found somewhat later (6-8 weeks). The changes observed were found in animals of different groups at the same time, but their number and intensity depended on the dose of NDMA.

The first tumors were recorded at about the same time (7.5-9 weeks), but the mean latent period of their onset differed in the different groups (Table 1). Differences in latent period were statistically significant ($P < 0.01$) between groups two and four, three and four, and one and three, but not significantly between groups one and two ($P > 0.05$).

The morphology of the neoplastic changes was described in detail previously [4]. Tumors of the liver were typical adenomas with large eosinophilic cells, forming round nodules compressing the normal tissue. Most of the liver tumors were hepatocellular carcinomas, characterized by intramural invasive growth and by the presence of multiple foci of extremely polymorphic and hyperchromic cells. Meanwhile giant cells with eosinophilic cytoplasm and a large nucleus were frequently observed, together with numerous mitotic figures. Besides changes in the hepatocytes, lesions also were observed in the hematopoietic tissue and were classified as hemocytoblastosis. Multiple foci of proliferation of small atypical basophilic cells were located mainly along the blood vessels in the liver, kidneys, and spleen; the structure of these organs was practically completely obliterated because of proliferation of blood cells. Adenocarcinomas of the kidneys developed from the epithelium of the tubules. Eosinophilic cells of different shapes and sizes formed adenomatous and (or) cystic structures. As a rule invasive growth was observed, but no metastases of the tumors in the kidneys (just as in the liver) were noted.

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TABLE 1. Induction of Tumors in *Rana temporaria* Treated with Various Concentrations of NDMA

Group of animals	Number of animals	NDMA concentration, ppm	Time of discovery of first tumor, weeks	Duration of exposure and experiment, weeks	Mean latent period of induction of tumors, weeks	Frequency of tumors*	Iball's index
1	65	1	8	30	22,1±6,2	8/54 (15%)	10
2	110	5	9	29	18,2±1,6	19/43 (44%)	35
3	62	10	7,5	16	13,0±0,5	22/31 (71%)	78
4	48	20	7,5	11	9,4±0,4	7/9 (78%)	118

*Ratio of number of animals with tumors to number of animals surviving until discovery of the first tumor.

In eight of the 54 frogs of group one which survived until discovery of the first tumor, nine neoplasms were discovered (one hepatocellular carcinoma, five adenomas, two cases of hemocytoblastosis, and one adenocarcinoma of the kidney), in 19 of the 43 frogs of group two 22 neoplasms were found (two hepatoadenomas, 15 hepatocellular carcinomas, and five cases of hemocytoblastosis). In group three, in 22 of the 31 animals 30 tumors were found (one hepatoadenoma, 20 hepatocellular carcinomas, six cases of hemoctyoblastosis, and three adenocarcinomas of the kidney), whereas in seven of the nine animals of group four there were 12 tumors (seven hepatocellular carcinomas, three cases of hemocytollastosis, and two adenocarcinomas of the kidney). The number of tumors per tumor-bearing animal was 1.13 in group 1, 1.16 in group two, 1.36 in group three, and 1.71 in group four ($P > 0.05$). Differences in the frequency of the tumors were significant between groups one and two, two and three, and two and four ($P < 0.01$), but not between groups three and four ($P > 0.05$). It is possible to compare both carcinogenic activity and the susceptibility of the animals by the use of Iball's index [3], which is determined by the formula:

$$\frac{\text{Percentage of animals with tumors}}{\text{Mean latent period (in days)}} \cdot 100.$$

This index differed for all the experimental groups and depended on the dose of NDMA (Table 1).

In the 270 intact control frogs no neoplastic changes were found during 56 weeks of observation in the liver, kidneys, and hematopoietic system; this is in agreement with data in the literature [2] on the absence of any such spontaneous tumors in this species.

The mean latent period of tumor development in amphibians and the frequency of appearance of tumors thus depend directly on the dose of the carcinogen. These findings reflect the general biological rules for tumor processes and they indicate that *Rana temporaria* can be used as a model for cancer research.

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