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Evaluating the Utility of N-Methyl-N-Nitrosourea as a Positive Control in Carcinogenicity Studies in the Albino Rats

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Abstract: The purpose of this study was to characterize the carcinogenic potential of N-methyl-N- nitrosourea (MNU), a DNA alkylating agent, in rodents to determine its suitability as a positive control agent in an alternative carcinogenicity model. A 60 total of Albino wistar rats (30 male and 30 female) were administered a single oral dose of 90 mg/kg and maintained for up to 13 weeks. Treatment was generally well tolerated; however, 30 rats died before completing the research due to neoplasms. MNU-related macroscopic observations of changes in some vital organs in the body of the rats were made, which correlated with the diagnosis of lymphoma of the hematopoietic system, noted in the thymus of all affected animals and the spleen, liver, lungs, and kidneys. The increased incidence of neoplastic and proliferative changes in MNU-treated rats suggests MNU could serve as a positive control in alternative carcinogenicity studies conducted in knockout rats.

Keywords: Carcinogenicity, MNU, Neoplasms, N-methyl-N-nitrosourea Albino Wistar rats.

1. Introduction

Studies of bioassays in rodents to evaluate carcinogenic risk associated with exposure to chemicals and drugs have been carried out. These bioassays have been used successfully by the National Toxicology Program (NTP) and others to create a large database of carcinogen testing information and have gained a high degree of credibility with regulatory agencies worldwide (National Toxicology Program 1979). These conventional assays are typically carried out using Albino Wistar rats,. In the past several years, transgenic mice that are genetically predisposed to cancer have become available, and efforts have been made to exploit these strains in alternative carcinogenicity studies (Tennant *et al*. 1999). In 1996, the International Conference on Harmonization (ICH) recognized that the conventional 2-year rodent bioassays had little or no relevance in predicting human cancer risk assessment based on the lack of correlation among positive findings in these bioassays to human cancer risk (MacDonald *et al*. 2004). In addition, this group acknowledged the utility of alternative carcinogenicity models for use in human cancer risk assessment by proposing that one of the 2-year rodent bioassays be complemented with a short-term (i.e., 6-month) study in one of the recently developed alternative mouse carcinogenicity models.

Compared to that of conventional 2-year bioassays, this shortened testing period has obvious advantages in reduced resources, earlier results, and a negligible background rate for

spontaneous tumors in these strains. However, a comprehensive set of data demonstrating the predictability of these animal models for carcinogenicity is not available; hence, there is some hesitancy in going forth with the use of these models to chemical entities (MacDonald *et al*. 2004). To further understand the predictability of these animal models, the International Life Sciences Institute (ILSI) in conjunction with the Health and Environmental Science Institute (HESI) coordinated a large-scale collaborative research program involving worldwide industrial, government, and academic laboratories to evaluate several models proposed for use in carcinogenicity assessment (Robinson and MacDonald 2001). The ILSI HESI results confirmed that a few of the alternative carcinogenicity models had better concordance with human cancer incidence than the lifetime rodent bioassays (Alden, Smith, and Morton 2002). Specifically, the transgenic mouse model could identify genotoxic carcinogens in a more accelerated manner than the standard 2-year rodent bioassays.

However, this study aims to evaluate N-methyl-N- nitrosourea (a DNA alkylating agent) as a potential carcinogenic material found in preservative foods using Albino Wistar rats as experimental animals .

3. Materials and Methods

3.1 Animals

Male and female (n = 15/sex/group) heterozygous Albino Wistar rats, approximately 8 to 9 weeks old and weighing approximately 20 g, were obtained from the National Veterinary Research Institute (Vom near Jos in Plateau State of Nigeria). Rats were housed in suspended, stainless-steel, wire-bottom cages. Environmental controls for the animal rooms were set to maintain a temperature of 64◦C to 79◦C, a relative humidity of 30% to 70%, and a 12-h light/12-h dark cycle. Diet of vital feed of appropriate nutrient i.e. nutrient of approximate amount bought in Gusau, Zamfara State of Nigeria, were used in the entire study and the institution's tap water treated by reverse osmosis were supplied ad libitum throughout the duration of the study. Animals were randomized to treatment groups by random number generation. To reduce the number of animals used in the study, wild-type controls were not included in the study design.

3.2 Treatments and Observations

MNU was obtained from Sigma Aldrich and prepared in 0.04 M citrate-buffered saline (pH 4.5) at 9 mg/ml on the day of use. Rats were administered a single dose of 90 mg/kg of MNU (10 ml/kg) in citrate-buffered saline (pH 4.5) by oral gavage (specified as day 1) within 2 h after the preparation of the formulation. Stability analysis was not conducted; however, given the robust response observed, one can presume a sufficient amount of intact MNU was present in the solution. Control rats received a single oral dose of citrate-buffered saline (pH 4.5) only.

In-life data collected for 13 weeks included clinical observations, body weight, food consumption, and mass palpation. At termination (days 92 and 93), observations included clinical pathology, macroscopic and microscopic observations, and organ weights. Hematology parameters evaluated included hemoglobin concentration, hematocrit, red and white blood cells, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets, and red cell distribution width.

3.3 Histopathological Evaluation

Tissues collected for histopathological evaluation were fixed in 10% phosphate-buffered formalin, except for the eyes and optic nerves and the testes and epididymides, which were fixed in Bouin's solution. Tissues were subsequently embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin for histopathological examination. The following tissues were examined: adrenal glands, aorta, brain (forebrain, midbrain, and hindbrain), cecum, colon, duodenum, esophagus, eyes and optic nerves, bone marrow glands, heart, ileum, gallbladder, kidneys, liver, lungs with bronchi, lymph nodes (mesenteric and mandibular), nasal cavity, ovaries, pancreas, peripheral nerve, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin with mammary glands, (cervical, thoracic, and lumbar), spleen, stomach (glandular and non-glandular), testes, thymus (thymic area), thyroid and parathyroid glands, tongue, trachea, tumors/masses, urinary bladder. The animal identification site and head were collected but not examined. In addition, gross lesions were similarly collected, processed, and examined by light microscopy.

Table 1: Comparison of mean body weights

DAYS	DOSE (mg/kg)			
	MALE		FEMALE	
	0	90	0	90
1	25.58	25.02	19.69	20.65
8	25.77	22.39	20.43	20.59
15	26.40	24.30	21.21	21.17
22	27.14	24.99	22.05	22.16
29	27.61	26.25	22.15	23.19
36	28.40	26.92	22.12	23.35
43	28.66	27.03	22.45	23.19
50	28.90	27.43	22.49	23.24
57	29.50	27.89	22.80	23.44
64	30.01	28.21	23.07	23.52
71	30.49	28.13	23.07	23.52
78	30.47	27.91	23.17	23.89
85	31.03	28.39	23.76	24.16
92	31.65	28.27	23.84	24.05

Table 2: Comparison of mean hematology data

MALE FEMALE		90	0	90
Hematocrit (%)	42.88	43.13	44.95	38.93
Hemoglobin (g/dl)	13.24	12.77	13.58	11.63
Red blood cells (×106/mm3)	8.576	8.990	9.143	7.818
Mean cell volume (µ3)	50.08	47.93	49.20	49.60
Mean cell hemoglobin (pg)	15.64	14.20	14.85	14.88
Mean cell hemoglobin (g/dl)	31.22	29.63	30.23	30.00
White blood cells (×103/mm3)	3.944	7.037	3.260	2.183
Neutrophils (%)	9.68	28.63	12.03	17.10
Lymphocytes (%)	85.82	64.23	83.88	79.10
Monocytes (%)	2.54	4.50	2.65	2.75
Red cell distribution width (%)	13.10	13.23	13.40	14.58

Table 3: Comparison of mean clinical chemistry data

	MALE		FEMALE	
	0	90	0	90
Blood urea nitrogen (mg/100 ml)	30.52	28.79	29.00	28.90
Aspartate aminotransferase (IU/l)	169.8	476.8	80.1	270.1
Creatinine (mg/100 ml)	0.090	0.067	0.075	0.093
Total bilirubin (mg/100 ml)	0.20	0.18	0.22	0.20
Glucose (mg/100 ml)	229.6	210.5	205.7	225.0
Potassium (mEq/L)	5.217	5.484	5.112	5.558
Alanine aminotransferase (IU/L)	149.3	250.6	48.3	119.3
Sodium (mEq/L)	153.50	154.80	153.92	154.53
Total protein (mg/100 ml)	4.98	4.84	4.93	4.85

Albumin (g/100 ml)	3.61	3.32	3.71	3.47
Globulin (g/100 ml)	1.38	1.30	1.29	1.18
Albumin/globulin ratio	2.697	2.604	2.914	2.985
Calcium (mg/100 ml)	8.73	8.98	8.92	9.04
Chloride (mEg/l)	111.23	113.56	114.33	115.25

4. Result and Discussion

Rationale for Dose Administered and Route of Exposure Oral administration was chosen for these studies because it was considered the most appropriate route of administration for comparison to studies evaluating novel agents that would have an oral route for exposure. The dose selected was based on previous studies where MNU was given by the intraperitoneal route. Administration of a single intraperitoneal dose of 75 mg MNU in the rats resulted in an increased incidence of skin and stomach hyperplasia and papillomas, forestomach squamous cell carcinoma, lymphoma, granulocytic leukemia, angiosarcoma, and duodenum adenomas in male and female rat 14 weeks after administration (Yamamoto *et al*. 1996). The administration of 90 mg MNU by oral gavage produced approximately equivalent DNA damage in the liver of rats to that produced by a single intraperitoneal dose of MNU (ILSI HESI Study 1997). DNA alkylation by MNU is considered to be a critical factor in NMU-induced tumorigenesis (Frei *et al.* 1978). Therefore, an oral dose of 90 mg was considered to be comparable to an intraperitoneal dose of approximately 75 mg, which is similar to doses used in multiple studies evaluating the effects of MNU.

5. In-Life Findings

MNU treatment was generally well tolerated; however, 6 (two males and four females) of 30 rats died between days 75 and 92 due to neoplasms. Specifically, the cause of death for the male rats may be attributed to a leiomyosarcoma noted in the non-glandular stomach (Daniel *et al*., 2008); the 3 female mice were diagnosed as having lymphoma in multiple tissues, including the liver, heart, kidney, lung, heart, and bone marrow. Treatment-related clinical observations consisted of decreased activity, alterations in breathing patterns (labored, rapid, or noisy), dehydration, hunched posture, coolness to the touch (data not shown). Symptoms of dehydration were observed in a few rats probably may be the resultant effect of NMU. Other clinical signs were evident beginning on day 63 or later. The total number of animals exhibiting

clinical signs at any time was 3 of 15 males and 4 of 15 females. There were no palpable masses noted by clinical observation. Body weight data are shown in Table 1. For the mice treated with MNU, both male and female rats had lost weight in the first week of the study compared to the first day of dosing. Both control and MNU-treated rats generally gained weight thereafter throughout the study. However, the week 1 weight loss effect persisted in the MNU-treated males, such that there was a slight decrease in body weights at the end of the study for males (11.5%); terminal female body weights were similar to the controls.

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