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BIOASSAY FOR CARCINOGENICITY OF 3,2′-DIMETHYL-4-NITROSOBIPHENYL, *O*-NITROSOTOLUENE, NITROSOBENZENE AND THE CORRESPONDING AMINES IN SYRIAN GOLDEN HAMSTERS*

STEPHEN S. HECHT, KARAM EL-BAYOUMY, ABRAHAM RIVENSON and EMERICH S. FIALA

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY 10595 (U.S.A.)

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SUMMARY

3,2′-Dimethyl-4-aminobiphenyl and 3,2′-dimethyl-4-nitrosobiphenyl were administered by subcutaneous injection in peanut oil to 2 groups of 15 male and 15 female Syrian golden hamsters. The total dose of each compound was 5.6 mmol/kg. In the group treated with 3,2′-dimethyl-4-aminobiphenyl, 24 animals had bladder tumors. In the group treated with 3,2′-dimethyl-4-nitrosobiphenyl 25 animals had subcutaneous tumors and 2 had bladder tumors. These results indicate that 3,2′-dimethyl-4-nitrosobiphenyl is a potent locally acting carcinogen. Total doses of 99 mmol/kg of aniline, *o*-toluidine, nitrosobenzene, or *o*-nitrosotoluene administered by subcutaneous injection failed to induce tumors.

INTRODUCTION

3,2′-Dimethyl-4-aminobiphenyl is a versatile carcinogen which induces bladder tumors in Syrian golden hamsters, tumors of the colon in male F344 rats, and mammary tumors in female F344 rats [3,8,14–17]. N-Oxidation, an established metabolic pathway for this compound, is likely to be involved in its activation [13]. The potent mutagenicity of 3,2′-dimethyl-4-nitrosobiphenyl in *Salmonella typhimurium* is in agreement with this hypothesis [5]. These results prompted us to carry out a comparative study

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of the carcinogenic activities of 3,[illegible]-dimethyl-4-aminobiphenyl and 3,2'-dimethyl-4-nitrosobiphenyl in Syrian golden hamsters. In a parallel assay, we tested the carcinogenicity in Syrian golden hamsters of the related single ring compounds aniline, *o*-toluidine, nitrosobenzene and *o*-nitrosotoluene. Aniline, *o*-toluidine and *o*-nitrosotoluene have previously been shown to induce tumors in rats [4,11,12,18].

MATERIALS AND METHODS

3,2'-Dimethyl-4-aminobiphenyl, aniline, *o*-toluidine, nitrosobenzene and *o*-nitrosotoluene were obtained commercially and purified as previously described [5]. 3,2'-Dimethyl-4-nitrosobiphenyl was synthesized from 3,2'-dimethyl-4-aminobiphenyl as described [5]. All compounds were pure according to analysis by gas chromatography and thin-layer chromatography.

Outbred Syrian golden hamsters were obtained at age 6 weeks from Simonsen Laboratories, Gilroy, CA. At 8 weeks of age they were divided into 16 groups of 15 animals each. Odd numbered groups were males and even-numbered groups were females. In each case the vehicle was peanut oil and all administrations were by subcutaneous injection. The animals were treated as follows: groups 1 and 2, 37 weekly injections of 0.15 mmol/kg of 3,2'-dimethyl-4-aminobiphenyl in peanut oil; groups 3 and 4, 37 weekly injections of 0.15 mmol/kg of 3,2'-dimethyl-4-nitrosobiphenyl; groups 5 and 6, 37 weekly injections of vehicle only; groups 7 and 8, 52 weekly injections of 1.9 mmol/kg of aniline oil; groups 9 and 10, 52 weekly injections of 1.9 mmol/kg of nitrosobenzene in peanut oil; groups 11 and 12, 52 weekly injections of 1.9 mmol/kg of *o*-toluidine; groups 13 and 14, 52 weekly injections of 1.9 mmol/kg of o-nitrosotoluene; groups 15 and 16, 52 weekly injections of vehicle only. After the injections were complete, animals were observed until moribund. Animals were housed 3 to a cage and allowed free access to NIH-07 diet and water. Cages were solid bottom polycarbonate and contained hardwood chip bedding. Laboratories were maintained at 21 ± 1°C and 50 ± 10% relative humidity. Animals were kept on light-dark cycles of 12-h duration starting at 07:00 h.

The experiment was terminated after 87 weeks. Upon necropsy, gross lesions and representative samples of all major organs were fixed in 10% buffered formalin and processed for microscopic evaluation.

RESULTS

Weights of control males increased from 110 g to 190 g in weeks 1–19, ranged from 190 g to 175 g in weeks 20–43, and from 175 g to 160 g in weeks 44–87. The corresponding values for control females were 110–210 g in weeks 1–20 and 210–180 g in weeks 21–87. With the exception of the animals treated with 3,2'-dimethyl-4-aminobiphenyl (groups 1 and 2), and aniline (groups 7 and 8), weights in treated groups were similar to controls.

Hamsters in group 1 reached a maximum mean weight of 160 g after 17 experimental weeks and in group 2, 185 g after 15 weeks. Weights remained constant until 50 weeks and then declined. Animals in group 7 reached a maximum mean weight of 160 g after 20 experimental weeks and in group 8, 170 g after 21 weeks. These weights were maintained throughout the experiment.

Mean survival times were shorter in most of the treated groups than in controls. The survival data for the biphenyl derivatives are summarized in Table 1. Among the single ring compounds, mean survival times were as follows: aniline, 67.7 weeks (group 7) and 62.1 weeks (group 8); nitrosobenzene, 60.8 weeks (group 9) and 52.5 weeks (group 10); *o*-toluidine, 61.3 weeks (group 11) and 57.8 weeks (group 12); *o*-nitrosotoluene, 45.4 weeks (group 13) and 51.1 weeks (group 14); controls, 75.5 weeks (group 15) and 68.7 weeks (group 16).

Tumors in groups 1–6 are summarized in Table 1. 3,2′-Dimethyl-4-aminobiphenyl induced bladder tumors in a high percentage of the animals. 3,2′-Dimethyl-4-nitrosobiphenyl was a powerful subcutaneous carcinogen, producing tumors at the site of injection in 28 of the 30 treated animals. Two animals presented with bladder papillomas. The incidence of tumors in the groups treated with the single ring derivatives (groups 7–14) was not significantly different from that in control hamsters.

DISCUSSION

The results of this study clearly demonstrate that 3,2′-dimethyl-4-nitrosobiphenyl is a powerful locally acting carcinogen in the subcutaneous tissue of the Syrian golden hamster. A structurally related compound, 2-nitrosofluorene, induced local sarcomas as well as breast tumors upon subcutaneous injection in rats [10]. The initiation of tumors by 3,2′-dimethyl-4-nitrosobiphenyl might be due to direct reaction with DNA or, as seems more likely, to reduction to the corresponding hydroxylamine with subsequent nitrenium ion formation. Nitrosoaromatics are known to undergo both non-enzymatic and enzymatic reduction to the corresponding hydroxylamines [1,6]. In contrast 3,2′-dimethyl-4-aminobiphenyl did not induce any subcutaneous tumors but rather gave a high yield of bladder tumors, as observed in previous studies [3,15]. Apparently, 3,2′-dimethyl-4-aminobiphenyl is not extensively N-oxidized in the subcutaneous tissue of the hamster. However, its metabolites, including the *N*-glucuronide of *N*-hydroxy-3,2′-dimethyl-4-aminobiphenyl, are excreted to a significant extent in hamster urine [13]. This *N*-glucuronide can be deconjugated under the acidic conditions of hamster urine, giving rise to *N*-hydroxy-3,2′-dimethyl-4-aminobiphenyl [13]. Based on the results of the present bioassay, the latter would be expected to be carcinogenic to the bladder epithelium [7].

In contrast to the potent tumorigenicity of the biphenyl derivatives, none of the single ring aromatic amines or C-nitroso compounds induced tumors

TABLE 1

NUMBER OF SYRIAN GOLDEN HAMSTERS WITH TUMORS IN GROUPS TREATED WITH 3,2'-DIMETHYL-4-AMINOBIPHENYL, 3,2'-DIMETHYL-4-NITROSOBIPHENYL AND VEHICLE CONTROL[a]

Group and No. of animals	Sex	Mean survival time (weeks)	No. of animals with								Other tumors
			Bladder tumors			Liver adenoma	Mammary adenocarcinoma	Subcutaneous tumors			
			Transitional cell carcinoma	Squamous cell carcinoma	Papilloma			Soft tissue pleiomorphic sarcoma	Rhabdomyosarcoma	Spindle cell sarcoma	
1. 3,2'-Dimethyl-4-aminobiphenyl 15(15)[b]	M	63	14	0	0	3	0	0	0	0	5[c]
2. 3,2'-Dimethyl-4-aminobiphenyl 15(14)	F	47	8	2	0	0	3	0	0	0	5[d]
3. 3,2'-Dimethyl-4--nitrosobiphenyl 15(15)	M	49	0	0	1	0	0	2	10	0	3[e]
4. 3,2'-Dimethyl-4--nitrosobiphenyl 15(14)	F	49	0	0	1[f]	0	0	1	4	9	0
5. Peanut oil 15(14)	M	66	0	0	0	0	0	0	0	0	1[g]
6. Peanut oil 15(14)	F	70	0	0	0	0	0	0	0	0	2[h]

[a]Each group consisted of 15 male or 15 female Syrian golden hamsters. Beginning at age 8 weeks, each animal received a subcutaneous injection of 0.15 mmol/kg of test compound in peanut oil once weekly for 37 weeks. Animals were observed until moribund. The experiment was terminated after 87 weeks.

[b]Number in parentheses is number of animals autopsied.

[c]1 stomach papilloma, 1 adrenal cortex adenoma, 1 esophageal carcinoma, 2 bladder leiomyosarcoma. Six animals had multiple tumors as follows: bladder carcinoma, stomach papilloma, liver adenoma; bladder carcinoma, esophageal carcinoma; bladder leiomyosarcoma, liver adenoma; bladder carcinoma, bladder leiomyosarcoma; bladder carcinoma, liver adenoma; bladder carcinoma, adrenal cortex adenoma.

[d]2 adrenal cortex adenoma, 1 uterus adenocarcinoma, 1 uterus leiomyoma, 1 soft tissue angiosarcoma. Two animals had multiple tumors as follows: bladder carcinoma, adrenal cortex adenoma, mammary adenocarcinoma, soft tissue angiosarcoma; bladder carcinoma, adrenal cortex adenoma, uterus leiomyoma.

[e]1 adrenal cortex adenoma, 2 soft tissue anaplastic tumors. One animal had an adrenal cortex adenoma, rhabdomyosarcoma, and bladder papilloma.

[f]One animal had a bladder papilloma and a spindle cell sarcoma.

[g]1 sarcoma with giant cells.

[h]1 uterus adenocarcinoma and 1 lymphoma.

under our experimental conditions although they did affect survival rates. Both *o*-toluidine and *o*-nitrosotoluene are carcinogenic in the F-344 rat, when administered in the diet, albeit at higher doses than in the present study [4,12,18]. High levels of aniline in the diet have been shown to cause tumors of the spleen in F344 rats [11]. Whether the present results are a reflection of the lower doses used, of the route of administration, or of a possible species difference requires further study. The lower activities of the single ring compounds than of the biphenyl derivatives are consistent with results of previous bioassays in rats and with mutagenicity data in *S. typhimurium* [2,5]. Two factors which could be responsible for their lower biological activities are lesser charge stabilization of a nitrenium ion conjugated with a single ring system rather than with a biphenyl system and/or a lesser effect on miscoding of a single ring arylamine DNA adduct compared to a bulkier aminobiphenyl adduct [2,9].

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REFERENCES

1 Becker, A.R. and Sternson, L.A. (1980) Non-enzymatic reduction of nitrosobenzene to phenylhydroxylamine by NAD(P)H. Bioorg. Chem., 9, 305–312.
2 Clayson, D. and Garner, R. (1976) Carcinogenic aromatic amines and related compounds. In: Chemical Carcinogens. American Chemical Society Monograph, 173, pp. 366–461. Editor: C.E. Searle. Washington, DC.
3 Fiala, E.S., Weisburger, J.H., Katayama, S., Chandrasekaran, V. and Williams, G.M. (1981) The effect of disulfiram on the carcinogenicity of 3,2'-dimethyl-4-aminobiphenyl in Syrian golden hamsters and rats. Carcinogenesis, 2, 965–969.
4 Hecht, S.S., El-Bayoumy, K., Rivenson, A. and Fiala, E. (1982) Comparative carcinogenicity of *o*-toluidine hydrochloride and *o*-nitrosotoluene in F344 rats. Cancer Letters, 16 103–108.
5 Hecht, S.S., El-Bayoumy, K., Tulley, L. and LaVoie, E. (1979) Structure mutagenicity relationships of N-oxidized derivatives of aniline, *o*-toluidine, 2'-methyl-4-aminobiphenyl, and 3,2'-dimethyl-4-aminobiphenyl. J. Med. Chem., 22, 981–987.
6 Horie, S. and Ogura, Y. (1980) Studies on the enzymatic reduction of C-nitroso compounds IV. Partial purification and kinetic properties of porcine heart C-nitroreductase. J. Biochem., 88 1141–1150.
7 Kadlubar, F., Flammang, T. and Unruh, L. (1978) The role of N-hydroxy arylamine N-glucuronides in arylamine-induced urinary bladder carcinogenesis: metabolite profiles in acidic, neutral and alkaline urines of 2-naphthylamine and 2-nitronaphthalene-treated rats. In: Conjugation Reactions in Drug Biotransformation, pp. 443–454. Editor: A. Aitio. Elsevier/North-Holland Biomedical Press, New York.
8 Katayama, S., Fiala, E., Reddy, B.S., Rivenson, A., Silverman, J., Williams, G.M. and Weisburger, J.H. (1982) Prostrate adenocarcinoma in rats: induction by 3,2'-dimethyl-4-aminobiphenyl. J. Natl. Cancer Inst., 68, 867–873.
9 Miller, E.C. (1978) Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidental Address. Cancer Res., 38, 1479–1496.

10 Miller, E.C., McKechnie, D., Poirier, M. and Miller, J.A. (1965) Inhibition of amino acid incorporation *in vitro* by metabolites of 2-acetylaminofluorene and by certain nitroso compounds. Proc. Soc. Exp. Biol. Med., 120, 538–541.
11 National Cancer Institute Carcinogenesis Technical Report Series No. 130 (1978) Bioassay of aniline hydrochloride for possible carcinogenicity. DHEW Publication No (NIH) 78-1385, Washington, DC.
12 National Cancer Institute Carcinogenesis Technical Report Series No. 153 (1979) Bioassay of *o*-toluidine hydrochloride for possible carcinogenicity. DHEW Publication No (NIH) 79-1709, Washington, DC.
13 Nussbaum, M., Fiala, E.S., Kulkarni, B., El-Bayoumy, K. and Weisburger, J.H. (1983) *In vivo* metabolism of 3,2′-dimethyl-4-aminobiphenyl (DMAB) bearing on its organotropism in the Syrian golden hamster and the F344 rat. Environ. Health Perspect., 49, 223–231.
14 Reddy, B.S. and Watanabe, K. (1978) Effect of intestinal microflora on 3,2′-dimethyl-4-aminobiphenyl-induced carcinogenesis in F344 rats. J. Natl. Cancer Inst., 61, 1269–1271.
15 So, B.T. and Wynder, E.L. (1972) Induction of hamster tumors of the urinary bladder by 3,2′-dimethyl-4-aminobiphenyl. J. Natl. Cancer Inst., 48, 1733–1735.
16 Spjut, H.J. and Spratt, Jr., J.S. (1965) Endemic and morphologic similarities existing between spontaneous colonic neoplasms in man and 3:2′-dimethyl-4-aminobiphenyl induced colonic neoplasms in rats. Ann. Surg., 161, 309–324.
17 Walpole, A.L., Williams, M.H.C. and Roberts, D.C. (1952) The carcinogenic action of 4-aminodiphenyl and 3:2′-dimethyl-4-aminodiphenyl. Br. J. Ind. Med., 9, 255–263.
18 Weisburger, E.K., Russfield, A.B., Homburger, F., Weisburger, J.H., Boger, E., VanDongen, C.G. and Chu, K.C. (1978) Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J. Environ. Pathol. Toxicol., 2, 325–356