Induction of ocular neoplasms in Fischer rats by intraocular injection of nickel subsulfide


Nickel subsulfide, $\alpha$Ni$_3$S$_2$, was administered to albino Fischer rats by a single injection into the vitreous body of the right eye (0.5 mg $\alpha$Ni$_3$S$_2$/rat, suspended in 20 $\mu$L of NaCl vehicle). Control rats received a similar injection of the vehicle. Malignant tumors developed in the injected eyes of 14/15 $\alpha$Ni$_3$S$_2$-treated rats by 8 months (vs. 0/11 controls, $p < 0.001$). Five of the injected eyes of $\alpha$Ni$_3$S$_2$-treated rats contained multiple tumors. The 21 eye tumors that were induced by $\alpha$Ni$_3$S$_2$ included 11 melanomas, four retinoblastomas, three gliomas, and three unclassified malignant neoplasms. Three of the melanomas developed extraocular extensions; one of the melanomas metastasized to lungs and brain. Although the melanomas arose from amelanotic uveal melanocytes, melanosomes were observed in electron micrographs of the tumor cells. This study provides a new experimental model for chemical induction of ocular neoplasms. As a procedure to test the carcinogenicity of nickel compounds, intraocular injection has the advantages of short latency period, high tumor incidence, and ease of tumor detection. (INVEST OPHTHALMOL VIS SCI 22:768-782, 1982.)

Key words: carcinogenesis, ocular tumors, nickel subsulfide, intraocular injection, melanoma, retinoblastoma, glioma

The carcinogenicity of nickel compounds in man and experimental animals has been reviewed in several articles and monographs. $^1$-$^6$ Nickel subsulfide, $\alpha$Ni$_3$S$_2$, is the most carcinogenic of numerous nickel compounds that have been tested in rats. $^7$-$^9$ Administration of $\alpha$Ni$_3$S$_2$ to rats by inhalation, $^10$ by implantation into heterotopic tracheas, $^11$ or by parenteral injections into muscle, $^6$ $^7$-$^9$, $12$, testis, $^13$ or kidney $^{14}$, $15$ results in malignant tumors at the sites of deposition. Exposure of cultured hamster embryo cells to $\alpha$Ni$_3$S$_2$ produces in vitro neoplastic transformation $^{16}$, $17$; the $\alpha$Ni$_3$S$_2$-transformed cells develop into undifferentiated sarcomas after subcutaneous inoculation in athymic (nude) mice. $^{18}$ As will be described in this article, administration of $\alpha$Ni$_3$S$_2$ to rats by a single intraocular injection induces intraocular malignant melanomas and retinoblastomas, and gliomas of the optic nerve. Short latency period, high tumor yield, and ease of tumor detection make this an attractive experimental system for research on metal carcinogenesis.

Table I. Induction of ocular tumors in rats by intraocular injection of $\alpha$Ni$_3$S$_2$

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Ocular neoplasms</th>
<th>Origin</th>
<th>Extraocular invasion</th>
<th>Distant metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>aNi$_3$S$_2$-Treated rats</td>
<td></td>
<td>Ciliary body</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>1</td>
<td>Melanoma (epithelioid)*</td>
<td>Ciliary body</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>Melanoma (spindle cell)</td>
<td>Iris</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>No tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Glioma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Melanoma (epithelioid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Retinoblastoma*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Melanoma (spindle cell)</td>
<td>Gliary body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Retinoblastoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Melanoma (spindle cell)*</td>
<td>Gliary body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Glioma</td>
<td>Retaina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Unclassified neoplasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Melanoma (mixed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Melanoma (mixed)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Glioma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Unclassified neoplasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td></td>
<td>Ciliary body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-20</td>
<td>No neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Diagnosis confirmed by transmission electron microscopy.

Materials and methods

**Test compound.** Nickel subsulfide ($\alpha$Ni$_3$S$_2$, median particle diameter 1.3 $\mu$m) was provided by INCO Ltd., Toronto, Ontario, Canada. The $\alpha$Ni$_3$S$_2$ was analyzed for aluminum, cobalt, chromium, copper, and manganese by emission spectroscopy (performed by Dr. Stuart Warner, Gordon Research Laboratory, INCO Ltd., Clarkson, Ontario, Canada); contamination by each of these metals was found to be less than 0.01% by weight. The identity of $\alpha$Ni$_3$S$_2$ crystals was confirmed by x-ray diffractionmetry (performed by Dr. Edvard Kostiner, Institute of Materials Science, University of Connecticut, Storrs, Conn.).

**Experimental animals.** Albino rats of the Fischer-344 strain (Charles River Breeding Laboratories, Inc., North Wilmington, Mass.) were housed in polystyrene cages, two to three rats per cage, and were fed laboratory rat Chow (Ralston-Purina Co., St. Louis, Mo.) and tap water ad libitum. At the time of intraocular injection, the rats were approximately 4 weeks old; their body weights averaged 52 gm (range 41 to 62 gm). The control group contained 11 males, and the $\alpha$Ni$_3$S$_2$-treated group contained 14 males and one female.

**Intraocular injection.** Each rat was anesthetized with diethyl ether. An injection into the vitreous cavity of the right eye was performed with a tuberculin syringe and No. 30 gauge needle under 10X magnification with a Kepler operating loupe. Each rat in the control group received an intraocular injection of 20 $\mu$L of sterile NaCl solution (0.15 mol/L). Each rat in the $\alpha$Ni$_3$S$_2$-treated group received a similar intraocular injection of NaCl vehicle containing 0.5 mg of $\alpha$Ni$_3$S$_2$. The suspension of $\alpha$Ni$_3$S$_2$ in sterile NaCl solution (25 mg $\alpha$Ni$_3$S$_2$/ml) was continually agitated with a magnetic stirring apparatus to ensure that constant amounts of $\alpha$Ni$_3$S$_2$ were aspirated into the syringe. Despite this precaution, the $\alpha$Ni$_3$S$_2$ dosage could not be precisely controlled because of partial sedimentation of $\alpha$Ni$_3$S$_2$ particles within the lumen of the syringe.

**Examination of rats.** During the week after the intraocular injections, the rats’ eyes were inspected daily. Therethere the rats were weighed at biweekly intervals and were carefully examined for development of ocular tumors. With one exception, the rats were killed within 2 weeks after an ocular tumor was detected. Rats that did not
develop tumors were killed when the experiment was terminated at 42 weeks. Inhalation of diethyl ether was used to kill the rats.

Pathologic examinations. The rats were autopsied and both eyes were enucleated. The eyes and specimens of brain, heart, lungs, liver, spleen, and kidneys were fixed in neutral buffered formalin. At least three histologic sections of each eye were prepared, with the attempt to include the pupil and the optic nerve. For examination by light microscopy, two sections of each eye were stained with hematoxylin and eosin, the third section was stained by the periodic acid-Schiff reaction. Selected eye sections were stained by the Fontana reaction for melanin and by alcian blue stain for acid mucopolysaccharide. Histologic sec-
Fig. 2. Malignant melanoma of the choroid, mixed cell type. (H & E; x232.)

tions of the other organs were stained only with hematoxylin and eosin. Electron microscopy was performed on specimens of five ocular tumors, which were fixed in glutaraldehyde or formalin, postfixed in osmium tetroxide, embedded in epoxy resin for ultrathin sectioning, and stained with lead and uranyl acetates. A JEOL JEM 7 electron microscope was used for the ultrastructural examinations.

Results

During the week after the intraocular injections there was minimal inflammatory reaction at the injection sites in αNi₃S₂-treated and control rats. Black particles of αNi₃S₂ were seen floating in the vitreous humor of αNi₃S₂-treated rats, and small intraocular hemorrhages were noted in a few treated and control rats. An ocular tumor was first observed at 26 weeks after the intraocular injection of αNi₃S₂. This tumor grew rapidly and eventually replaced the entire orbit. When the rat was killed at 30 weeks after the injection, the tumor had invaded the cranium by extension along the optic nerve to the optic chiasm and had metastasized to the cerebrum and lung. Between 34 and 36 weeks after the intraocular injections, ocular neoplasms became clearly visible in 13 of 14 surviving αNi₃S₂-treated rats. The tumor-bearing rats were killed at 36 weeks so that the sites of tumor origin could be established. The sole surviving αNi₃S₂-treated rat and the 11 control rats were killed at 40 to 42 weeks after the intraocular injection.

The outcome of the carcinogenesis study is listed in Table I. Malignant ocular tumors were found in 14/15 injected eyes of αNi₃S₂-treated rats vs. 0/11 injected eyes of control rats (p < 0.001 computed by Fisher’s exact test). Five of the injected eyes of αNi₃S₂-treated rats contained multiple neoplasms. The 21 distinct ocular tumors that were identified by histologic examination included 11 melanomas, four retinoblastomas, three gliomas, and three unclassified malignant tumors.
Fig. 3. Malignant melanoma of the choroid, mixed cell type. Note calcification (arrow) and sclera (S). (H & E; ×93.)

neoplasms. Tumor size varied from lesions that filled the globe and extended into the orbit to tumors of 1 or 2 mm in largest diameter. No tumors were found in the noninjected (left) eyes of the αNi₃S₂-treated or control rats. No primary extraorbital tumors were observed in the αNi₃S₂-treated or control rats.

Melanomas. Criteria for the diagnosis of melanoma were as follows: (1) tumors arising from the uvea, consisting of spindle-shaped or epithelioid cells; (2) the spindle cells were
Fig. 4. Mixed spindle A and spindle B type melanoma cells. The cells show elongated cell bodies, elongated nuclei (N) and small nucleoli (nl). Endoplasmic reticulum (ER), free ribosomes (r) and scant mitochondria (m) are seen in the cytoplasm. (×12,550.)

cohesive cells containing spindle-shaped nuclei with ill-defined cell borders, resulting in the nuclei superficially appearing to be in a syncytium; (3) the more narrow spindle nuclei exhibited nuclear folds and the plumper spindle nuclei contained prominent nucleoli; (4) the epithelioid cells were noncohesive cells with distinct cell borders and large oval nuclei with prominent nucleoli. The 11 melanomas were observed in 10 eyes; one eye contained two separate melanomas involving the iris and ciliary body. Based on the
Fig. 5. Epithelioid-type melanoma cells, revealed rounded cell bodies, large nuclei (N), well-formed nucleoli (nl), numerous mitochondria (arrows), short branches of endoplasmic reticulum (ER), and free ribosomes (R). (×5655.)

Callendar classification of melanomas, there were five spindle-cell tumors, two epithelioid tumors (Fig. 1), and four mixed-cell tumors (Figs. 2 and 3). Two of the melanosomas arose from the iris, six from the ciliary body, and two from the choroid. Three melanosomas filled or destroyed the globe and extended into extraocular tissues; one melanoma metastasized to lungs and cerebrum. Transmission electron microscopy performed on four melanosomas confirmed the presence of combinations of the characteristic melanoma cell
Ocular tumors induced by Ni$_3$S$_2$

Fig. 6. Portion of an epithelioid melanoma cell, showing premelanosomes (P) and melanin granules (m). (×21,840.)

types: spindle A, spindle B (Fig. 4), and epithelioid (Fig. 5). Premelanosomes and melanosomes were frequently seen in the cytoplasm of the melanoma cells (Fig. 6). In paraffin sections the melanosomes stained positively by the Fontana reaction. The metastatic melanoma in the lung of rat No. 1 was predominantly of the epithelioid cell type.

Retinoblastomas. Criteria for the diagnosis of retinoblastoma were tumors arising from the retina composed principally of undifferentiated neuroblastic cells that contained
large hyperchromatic nuclei and scanty cytoplasm. The occurrence of mitotic figures and neuroepithelial rosettes was variable. The four retinoblastomas were similar in histologic appearance to that illustrated in Fig. 7. Electron microscopy of one of the retinoblastomas showed ultrastructural features characteristic of poorly differentiated retinoblastoma (Figs. 8 and 9).

**Gliomas.** Criteria for the diagnosis of astrocytomas ("gliomas") of the optic nerve were as follows: tumors containing any one or more of the three main histologic patterns, i.e. (1) reticulated or myxomatous, (2) "fibrous" or astrocytic, and (3) transitional. Three gliomas of the optic nerves were identified. Their histologic appearance (Figs. 10 and 11) resembled the astrocytic gliomas (hamartomas) seen in human tuberous sclerosis and neurofibromatosis.

**Unclassified tumors.** Three tumors were observed that were similar in histologic appearance. Although these tumors appeared to be malignant, they did not fit in the clinical categories of ocular tumors; the cells of origin could not be identified. These tumors filled the vitreous body and involved the retina. Electron microscopy of one of these tumors revealed cells with condensed chromatin along the nuclear envelope and large lipid vacuoles in the cytoplasm.

**Other ocular lesions.** Light microscopy of the injected eyes of αNi₃S₂-treated rats disclosed several benign hyperplastic and metaplastic lesions. The retinal pigment epithelium exhibited fibrous metaplasia in 5/15 eyes; the neural retina was dysplastic in 1/15 eyes. Hyperplasia of the ciliary body epithelium was noted in 1/15 eyes; ruberosis irides was seen in 2/15 eyes; corneal endothelial hyperplasia occurred in 1/15 eyes; and metaplastic cartilage was observed within the vitreous and lens of 1/15 eyes. Fibrous metaplasia and dysplasia of the lens epithelium...
Fig. 8. Retinoblastoma cells extending through the vitreous side of the internal limiting membrane (ILM) into the vitreous cavity nerve fiber layer is indicated at the arrow. The tumor cells have the characteristic findings of triple membrane structures (TMS) involving the nuclear envelope, large and small nucleoli (nl), rough endoplasmic reticulum (ER), numerous ribosomes (R), and cell attachments by zonula adherens (ZA) and macular occludens (arrow). (×6600.)
was seen in 8/13 lenses that were sectioned. Hyperplastic and metaplastic lesions were not observed in the injected eyes of control rats.

Various pathologic changes that were attributed to the trauma of intraocular injection were noted in the injected eyes of \( \alpha \text{Ni}_3\text{S}_2 \)-treated and control rats. These benign lesions included healed penetrating injury of the sclera, focal calcification, retinal gliosis, cataracts, vitreous organization, and vitreous hemorrhages.

**Extraocular lesions.** Peribronchial foci of mononuclear cells were noted in lungs of many rats in the \( \alpha \text{Ni}_3\text{S}_2 \)-treated and control groups, consistent with a diagnosis of endemic infectious pneumonitis. No other extraocular lesions were observed.

**Discussion**

Epidemiologic investigations of nickel refinery workers in Canada, Wales, Norway, U.S.S.R., and New Caledonia have revealed increased risks of cancers of the respiratory tract (i.e., carcinomas of the lung, nasal cavities, and in one study, larynx).\(^1\)\(^-\)\(^4\) These observations have established an association between exposures of workmen to inhalation of nickel-containing dusts or vapors and subsequent development of respiratory tract neoplasms.\(^5\)\(^-\)\(^6\) Nickel compounds, e.g., \( \alpha \text{Ni}_3\text{S}_2 \) and nickel carbonyl, \( \text{Ni(CO)}_4 \), have been shown to induce malignant tumors in rats after administration by inhalation or parenteral routes.\(^2\)\(^-\)\(^3\)\(^-\)\(^15\) Ocular tumors have not previously been identified in any clinical or experimental study of nickel carcinogenesis; the present report presents the first evidence that nickel compounds are carcinogenic for the eye.

The occurrence of ocular tumors in workers exposed to various chemicals has been discussed in recent articles by Albert et al.\(^2^2\) and Smith and Egan.\(^2^3\) These papers have
Fig. 10. Optic nerve glioma in fibrous area. (H & E; ×160.)

Fig. 11. Optic nerve glioma; myxomatous area with microcystoid spaces. (H & E, ×200.)
Table II. Induction of ocular tumors in experimental animals

<table>
<thead>
<tr>
<th>Authors</th>
<th>Animal</th>
<th>Route</th>
<th>Substance</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patz et al.</td>
<td>Mouse</td>
<td>Intraocular</td>
<td>20-Methylcholanthrene</td>
<td>Diverse tumors in 17/326 injected eyes</td>
</tr>
<tr>
<td>Benson</td>
<td>Rat</td>
<td>i.p.</td>
<td>Ethionine &amp; N2-AAF</td>
<td>Melanoma in 1/25 rats</td>
</tr>
<tr>
<td>Taylor et al.</td>
<td>Dog</td>
<td>i.v.</td>
<td>226Ra</td>
<td>Melanomas in 1/25 rats &gt;5 yr old*</td>
</tr>
<tr>
<td>Evgen'eva</td>
<td>Rat</td>
<td>Intraocular</td>
<td>Platinum foil</td>
<td>Melanomas in 1/55 dogs &gt;5 yr old</td>
</tr>
<tr>
<td>Present</td>
<td>Rat</td>
<td>Intraocular</td>
<td>aNi3S2</td>
<td>Diverse tumors in 14/15 rats</td>
</tr>
</tbody>
</table>

*Tumors were bilateral in 20% of dogs.

emphasized that ocular tumors may often be overlooked in epidemiologic surveys, even though tumors at extraocular sites are effectively identified. Animal models for study of ocular tumors have been reviewed by Albert et al. As summarized in Table II, there have been few previous reports of experimental induction of ocular tumors in animals by administration of chemical substances. In none of the earlier studies has the incidence of eye tumors approached the 93% incidence (14/15) observed in the present investigation.

Induction of ocular neoplasms by intraocular injection of aNi3S2 provides a new experimental system to investigate the molecular mechanisms of ocular carcinogenesis and to evaluate the efficacy of various modes for therapy of eye tumors. From a methodologic viewpoint, intraocular administration of aNi3S2 has the advantage that only a single dose of the chemical is necessary; the injection can be performed in less than 5 min. Localization of aNi3S2 can be controlled by visual inspection. For certain purposes, the opposite eye might be used as a control. Since the vitreous body is juxtaposed to the retina, uveal tract, lens, and optic nerve, injection of aNi3S2 into the vitreous body ensures exposure of diverse cell types to the carcinogen. Based on the results of the present study, the ocular tissues adjacent to the vitreous body are all susceptible to neoplastic transformation by aNi3S2. We speculate that intraocular injection might prove to be a useful technique for carcinogenesis testing of other nickel compounds, and, possibly, of other classes of chemicals.

A striking finding in the present experiments was the presence of melanosomes in some of the ocular melanomas arising from amelanotic uveal melanocytes. This is similar to the observation of Pawlowski et al., who observed that 7-12-dimethylbenzanthracene produced pigmented cutaneous melanomas in albino guinea pigs. The mechanism by which amelanotic melanocytes become capable of making melanin deserves further study.

The tumor latency period of 26 to 36 weeks that was observed in the present study is significantly shorter than the latency periods that were previously reported after administration of aNi3S2 to Fischer rats by intramuscular, intrarenal, intratesticular, or respiratory routes. The 93% incidence of malignant eye tumors that was obtained in the present study was substantially greater than the local sarcoma incidences of 23% and 77% that were previously observed in Fisher rats within 2 yr after intramuscular injections of 0.6 or 1.2 mg of aNi3S2, respectively. Partial explanations for the short latency period and high tumor yield in the present study may be deduced from the anatomy and pathophysiology of the vitreous body and its adjacent tissues. First, the vitreous body lacks a blood supply and is isolated from the systemic vascular system by the blood-retina barrier and the ciliary epithelium. Therefore Ni(II) ions, which are gradually released from aNi3S2 in vivo, are likely to be mobilized very slowly from the vitreous body. We infer that local concentrations of Ni(II) remain high for a protracted period after an intraocular injection of aNi3S2. Second, the local inflammatory reaction after intraocular injection of aNi3S2 is much milder than previously observed at other locations.

Downloaded From: https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933102/ on 11/23/2018
particles of αNi₃S₂ within the vitreous body are relatively sequestered from phagocytosis by macrophages. Third, cells that undergo malignant transformation in ocular tissues may be relatively protected from contact by immunocompetent cells responsible for tumor rejection at other sites. Fourth, smaller tumors can be detected in the right eye by palpation and visual inspection than can be identified in other internal locations such as lung, muscle, testis, or kidney.¹⁰⁻¹⁸ We speculate that interplay of these four factors may account for rapid tumor development and high tumor incidence in the rat eye after intraocular injection of αNi₃S₂.

We are grateful to Michael D. Wagener, M.D., Max Goodman, M.D., James L. Carpenter, D.V.M., Damon R. Averell, Jr., D.V.M., and Paul M. Newberne, D.V.M., Ph.D. for consultation in interpreting the histology slides, and to John M. Mitchell, B.S., and Russell Jacobsen, B.S., for skillful technical assistance.

REFERENCES.

25. Pawlowski A, Haberman HF, and Menon IA: Skin melanomas induced by 7,12-dimethylbenzanthra-