Hematologic malignancies developing in Syrian golden hamsters during induction of pancreatic carcinoma

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We report 30 hematologic malignancies arising in 25 of 236 Syrian golden hamsters (SGH) that received combinations of N-nitroso(2-oxopropyl)amine (BOP) and Streptozotocin (STZ). Lesions developed with morphological similarity to human small lymphocytic (n = 7), diffuse mixed (n = 2), diffuse large cell lymphoma (n = 12), follicular lymphoma (n = 2), anaplastic large cell lymphoma (n = 3), hairy cell leukemia (n = 2), malignant histiocytosis (n = 1) and discordant lymphomas (n = 5). The types and distribution of these lesions are different from epizootic lymphomas in SGH. We also report a higher percentage (12 versus 4.6%) and the earlier appearance (<40 versus 80–112 weeks) compared with aging-associated spontaneous SGH lymphoma. The features of these hematologic malignancies have not been previously reported in epizootic or aging-associated spontaneous lymphomas and therefore suggest a new class of hematologic lesions in SGH. Benign and atypical hyperplasia correlated with STZ administration (r = 0.97, P = 0.03). The malignant lesions correlated with areas of lymphoid hyperplasia (r = 0.78, P = 0.004). Only one of the 21 untreated SGH spontaneously developed a low grade lymphoma. The unusual types, distribution and occurrence of these lesions may suggest a role for these carcinogens in their induction.

Introduction

In a recently published pancreatic carcinogenesis study using N-nitros(2-oxopropyl)amine (BOP) and streptozotocin (STZ), we observed pancreatic carcinomas (1), and a high number of hematologic lesions among 236 Syrian golden hamsters (SGH) comprising the treated groups. BOP is a dicarboxy-N-nitosodipropylamine derivative which is a highly selective and potent pancreatic carcinogen in SGH and which is known to induce, to a much lesser extent, tumors of the lungs, liver, gallbladder, and urogenital tract (2). STZ, an N-methyl-N-nitrosourea derivative of glucosamine, is widely used experimentally for the chemical induction of insulin-deficient diabetes mellitus (3). Evidence also suggests that the diabetogenic effect results in perturbation of lymphocyte function (4–9). STZ has also been the subject of studies regarding its chemotherapeutic (10) properties in human tumors and oncogenic activity (11–13) in SGH. However, to date, no report suggests that STZ or BOP or its combination is associated with the development of hematologic malignancies.

Although SGH have the lowest rate of spontaneous neoplasms among rodents (14), hematologic malignancies, in particular malignant lymphomas, represent the most common spontaneous neoplasm in SGH (15,16). These lymphomas are either aging-associated lesions or epizootic types occurring in 2.3–4.6% of older SGH, developing by 80–112 weeks of age (17–19). Likewise, epizootic outbreaks of lymphomas have been reported, occurring early in 50–90% of young animals from isolated colonies of SGH. Such reports have suggested an underlying horizontally transmitted C-type or DNA viroid-like particle(s) (23), or more recently, a polyomavirus (24,25). The purpose of this report is to describe a unique group of hematologic lesions that differ from either epizootic or aging-associated spontaneous lymphomas in SGH and may be induced by STZ therapy. We report lesions that have a higher incidence and earlier appearance of tumors compared with that of aging-associated SGH lymphomas; lesser incidence and later appearance than epizootic types; and a wide variety of lesions that have not been previously reported in SGH. We observed a strong correlation between the presence of lymphoid hyperplasia and lymphoma; and between exposure to STZ and incidence of lymphoid hyperplasia. These data further support the controversial role of STZ in lymphoproliferation and lymphoid-mediated STZ diabetes.

Materials and methods

The experimental groups, dosage and treatment protocol are summarized in Table I and the experimental rationale is previously described in detail (1). Two hundred and fifty seven SGH that comprised the study were obtained from Simonsen Lab. (Gilroy, CA). All were male, 6 weeks old at the time of arrival, housed in plexiglass cages with wire tops, four animals per cage and divided into eleven experimental groups (Groups A to F).

During the 40 week study period, 50 premature deaths occurred. Autopsies were performed immediately. All surviving SGH were sacrificed and autopsied at 40 weeks. At the time of sacrifice, the pancreas, distal stomach, duodenum, proximal jejunum, spleen, surrounding peripancreatic soft tissues, and lymph nodes were excised in toto, fixed in Bouin’s solution and embedded in paraffin. Sections were cut on a microtome at 5 μm thickness and stained with hematoxylin and eosin. All sections were then coded with randomly drawn numbers and underwent histological examination by light microscopy in a blind fashion without knowledge of the experimental treatment given. Several cases underwent electron microscopic evaluation for C-type particles.

Sections from the 257 SGH were evaluated and the hematologic lesions of the lymph nodes, spleen and extralymphatic tissues (i.e. gastro-intestinal tract, pancreas, mesentery) were classified into malignant and hyperplastic processes using their histologic features (Table I). Of the 25 SGH with hematologic malignancies, 10 had concurrent pancreatic carcinomas (1). Hyperplastic hematologic processes include reactive and atypical hyperplasia. The remainder of the SGH without lesions showed normal histology. Most lesions were classified using the National Cancer Institute Working Formulation for The Classification of Non-Hodgkin’s Lymphoma (26), a classification which allows for histologic grading of lymphomas on the basis of histologic features. Some cases are classified into malignant and hyperplastic processes using their histologic features (Table I). Of the 25 SGH with hematologic malignancies, 10 had concurrent pancreatic carcinomas (1). Hyperplastic hematologic processes include reactive and atypical hyperplasia. The remainder of the SGH without lesions showed normal histology. Most lesions were classified using the National Cancer Institute Working Formulation for The Classification of Non-Hodgkin’s Lymphoma (26), a system which allows for histologic grading of lymphomas on the basis of morphology alone. Although, one lesion mimicked Hodgkin’s lymphoma, no diagnostic Reed-Sternberg cells were identified. We used the terminology of the Kiel classification for anaplastic large cell lymphoma (27).

The hematologic lesions were evaluated as to the type and histologic grade, the site and distribution. Statistical analysis was performed using McNemar (28) chi-square analysis with Yates correction for small values, correlation
Malignant hematologic lesions spontaneously developed a low grade lymphoma in the compared with the untreated group, although one hamster

A significant number of SGH developed malignant lesions compared with the untreated group, although one hamster spontaneously developed a low grade lymphoma in the

[Table I. Hematological findings in all groups]

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Malignantab</th>
<th>Hyperplasiac</th>
<th>Totald benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BOP at wk 0 only; SAC at wk 40</td>
<td>6/42 (14.3%) Large cleaved lymphoma (3), maligoma (1), diffuse mixed (2)</td>
<td>14/42 (33%)</td>
<td>36/42 (85.7%)</td>
</tr>
<tr>
<td>B</td>
<td>BOP at wk 0; STZ at wk 10; SAC at wk 40</td>
<td>9/40 (22.5%) Large anaplastic lymphoma (2), large cell (5), hairy cell, diffuse mixed</td>
<td>15/40 (37%)</td>
<td>31/40 (77%)</td>
</tr>
<tr>
<td>Bx</td>
<td>BOP at wk 0; SAC at wk 10</td>
<td>1/10 (10%) Hairy cell</td>
<td>3/10 (30%)</td>
<td>9/10 (90%)</td>
</tr>
<tr>
<td>C</td>
<td>BOP at wk 0; STZ at wk 20; SAC at wk 40</td>
<td>5/41 (12.2%) Follicular small cleaved, large cell (2), lymphoplasmacytic, malignant histiocytosis</td>
<td>16/41 (39%)</td>
<td>36/41 (87%)</td>
</tr>
<tr>
<td>Cx</td>
<td>BOP at wk 0; SAC at wk 20</td>
<td>0/9 (0%)</td>
<td>3/9 (33%)</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td>D</td>
<td>BOP at wk 0; STZ at wk 30; SAC at wk 40</td>
<td>5/41 (12.19%) Small lymphocytic (2), follicular, large cleaved (2), large anaplastic</td>
<td>10/41 (24%)</td>
<td>36/41 (87%)</td>
</tr>
<tr>
<td>Dx</td>
<td>BOP at wk 0; SAC at wk 30</td>
<td>0/8 (0%)</td>
<td>3/8 (37%)</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>E1</td>
<td>STZ at wk 10 only; SAC at wk 40</td>
<td>0/15 (0%)</td>
<td>11/15 (73%)</td>
<td>15/15 (100%)</td>
</tr>
<tr>
<td>E2</td>
<td>STZ at wk 20 only; SAC at wk 40</td>
<td>4/15 (26.6%) Small lymphoplasmacytic (2), maligoma, follicular small cleaved</td>
<td>8/15 (53%)</td>
<td>11/15 (73%)</td>
</tr>
<tr>
<td>E3</td>
<td>STZ at wk 20 only; SAC at wk 40</td>
<td>0/5 (0%)</td>
<td>3/5 (60%)</td>
<td>5/10 (50%)</td>
</tr>
<tr>
<td>E4</td>
<td>No treatment; SAC at wk 40</td>
<td>1/21 (4.7%) Diffuse small lymphocytic</td>
<td>3/21 (14%)</td>
<td>20/21 (95%)</td>
</tr>
<tr>
<td>Total</td>
<td>31/257 (12%)</td>
<td>389/257 (88%)</td>
<td></td>
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</tr>
</tbody>
</table>

*McNemar's chi-square test (with Yates correction) for malignant lesions: (i) total treated group (A-E1) versus untreated group (F), P < 0.0001; (ii) BOP treatment alone (A,B,C,Dx) versus untreated group (F), P < 0.0001; (iii) BOP followed by STZ treatment (B,C,D) versus untreated group (F), P < 0.0001; (iv) STZ treatment alone (E2) versus untreated group (F), P < 0.0001.

*Paired t-test showed significant correlation between malignant and hyperplastic lesions, r = 0.78, P = 0.0042.

*Significant correlation for all groups and lymphoid hyperplasia, r = 0.65, P = 0.02; and for STZ duration versus frequency of lymphoid hyperplasia, r = 0.97, P = 0.03; five of 257 have features of atypical hyperplasia. No significant correlation between BOP treatment and hyperplasia (P = 0.34) and between BOP/STZ treatment and hyperplasia (P = 0.07).

Total benign includes tissue with normal histology, lymphoid hyperplasia as well as those involved by extramedullary hematopoiesis BOP, N-nitrosobis(2-oxopropyl)amine; STZ, streptozotocin; SAC, scheduled sacrifice time.

Results

Survival and premature deaths

Of a total of 50 SGH that died prematurely during the study period, 26.0% (13/50) had malignant hematologic lesions, while only 6.7% (12/180) of SGH surviving the 40 week study period were found to have malignant hematologic lesions (P < 0.05). The mean survival of animals with malignant hematologic lesions (36.9 ± 4.5 weeks) did not significantly differ from the mean survival of animals without malignant hematologic lesions (38.0 ± 5.2 weeks). Nevertheless, 52% (13/25) of SGH that developed malignant hematologic lesions died prematurely during the course of the 40 week study period.

Correlation of lymphoid hyperplasia and lymphoma in all groups

A significant correlation existed between the length of exposure to STZ and the frequency of lymphoid hyperplasia (r = 0.97, P = 0.03) (Figure 1, Table I). There was also a significant correlation between the number of malignant lesions and the number of SGH with hyperplasia (r = 0.78, P = 0.0042) (Figure 3) for all treated groups.

In contrast, no significant correlation existed between BOP administration and hyperplasia (P = 0.34) or between hyperplasia and the group treated with BOP followed by STZ (P = 0.07). However, when one considers all the treated groups, the correlation of treatment with the frequency of hyperplasia then becomes significant (P = 0.02).

Malignant hematologic lesions

A significant number of SGH developed malignant lesions compared with the untreated group, although one hamster spontaneously developed a low grade lymphoma in the untreated group (Table I). The lymphomas were either pure in histologic type or consisted of a combination of lesions. Twenty animals had a single pattern of lymphoma and five had a doublet of discordant lymphoma. The histopathology was assigned to the closest human equivalent. Using the Working
Formulation and Kiel classification schemes, nine low grade, 15 intermediate grade and three high grade lesions were detected (Figure 2, panels A–H). In addition, two hairy cell leukemias and one malignant histiocytosis developed. Thus, we encountered a total of 30 malignant hematologic lesions in 25 SGH treated with BOP and/or STZ.

Distribution of lesions
Up to 80% showed splenic, 63% nodal and 16% gastrointestinal tract involvement. Eight (27%) lesions were extralymphatic (i.e. mesentery, pancreas, gastrointestinal tract) and 26 (86%) were nodal or splenic in location (Table II).

Discussion
Carcinogens predisposing animals to lymphoma development are rarely observed in animal models (29) including SGH. Chemical induction of lymphomas was first reported in 1959 in newborn Swiss mice injected with 7,12-dimethylbenz[a]anthracene (DMBA) (30). Most of the mice developed an undifferentiated (small non-cleaved) type of lymphoma. DMBA administered to AKR mice resulted in accelerated development of lymphoma starting at 10 weeks of age. However, the frequency of malignant lymphoma was similar in the treated group and untreated animals; a finding attributed
to the presence of an oncogenic virus (31). When DBMA was administered in SGH, only a few animals developed malignant lymphoma (32). To date, there has been no reported association of a chemical carcinogen or a combination of carcinogens including that of BOP or STZ with the development of lymphoma in SGH. BOP administration in SGH leads to the development of pancreatic carcinoma (1) attributed partly to K-ras oncogene activation (33). To our knowledge, BOP administration has not been associated with abnormalities of lymphoid organs or lymphocytes. Our results suggest no relation between BOP treated SGH and the frequency of hyperplastic or neoplastic hematologic lesions (Table I). Our results suggest that the observed hematologic lesions may be explained by STZ administration considering the known effect of STZ on lymphoproliferation (4–8).

This finding is not surprising in the light of recent literature on STZ. STZ, an antibiotic derived from Streptomyces achromogenes, is known to perturb lymphocyte properties and function in animal studies. Aberrations in lymphocyte metabolism (decreased products of glucose metabolism) (4) and alteration of phenotype (markedly increased CD5+ subset of lymphoid cells) (5) occur in STZ-induced diabetic rats. Subcutaneous injection of subdiabetogenic doses of STZ (to dissociate its putative direct toxicity to the islets) induced hyperplasia and enhanced [3H]thymidine incorporation in adjacent lymph nodes (6). STZ induces chromatid aberrations, cell killing and sister chromatid exchanges in Chinese hamster ovary cell line (34). In rats, thymic hypoplasia and maturational impairment of thymic lymphocytes (7) and in mice, impairment of bone marrow precursor T cells (8) are observed. Indeed, STZ administration significantly correlates with the frequency of lymphoid hyperplasia and malignant hematologic lesions in SGH (Figures 1, 3 and Table I).

If one may speculate, these carcinogens may also be inducing exogenous virus or putative endogenous-virus transforming abilities. Rare examples of viral and chemical co-carcinogenesis exist: the frequency of lymphoma increased in SGH infected with lymphocytic choriomeningitis virus and then treated with 7,12-dimethylbenz[a]anthracene (35) and in the induction of oral tumors in SGH infected with Herpes simplex virus (HSV) and given carcinogens (36).

Viruses that promote certain lymphomas in SGH with no known prior carcinogen exposure are also well described. In this regard, epizootic spontaneous lymphomas secondary to transmissible agents in colonies of SGH are well known (20–23). The explanation for the occurrence of epizootic lymphomas was however not clear but two processes were cited (20): (i) activation of an endogenous or exogenous virus by some undetermined carcinogen, and (ii) transfer of exogenous virus by direct or indirect contact including cannibalism. The latter explanation is unlikely in our study because our animals were isolated four to a cage. The former process cannot be ruled out in this study despite absence of C-type particles by electron microscopy (data not shown). Recently, a polyomavirus was documented as the putative etiology of an epizootic form of spontaneous lymphoma in SGH. The polyomavirus (HaPV) caused lymphomas when injected into newborn hamsters (24,25). This polyomavirus was isolated from skin epitheliomas of hamsters and when injected in newborn hamsters, viral replication preferentially occurred in lymphoid organs causing lymphomas. The lymphomas that developed were virus-free (37) but lympho-

![Fig. 3. Area graph showing the hyperplastic and malignant hematologic lesions seen in the treatment groups. There is a significant correlation between the frequency of hyperplastic lesions and malignant lesions within each treatment group.](https://academic.oup.com/carcin/article-abstract/17/9/1983/338055)

### Table II. Classes of hematological lesions in Syrian golden hamster

<table>
<thead>
<tr>
<th>Variable</th>
<th>Epizootic</th>
<th>Aging</th>
<th>Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>50–90% of the colony (23)</td>
<td>2.3–4.6% (16,17,38,39)</td>
<td>12%</td>
</tr>
<tr>
<td>Onset</td>
<td>Shortly after birth (23)</td>
<td>As early as 56 weeks, mean age of 92 weeks (40)</td>
<td>Close to 40 weeks</td>
</tr>
<tr>
<td>Distribution</td>
<td>45% in small intestine, rare in spleen (20)</td>
<td>Mostly mesenteric lymph nodes, spleen 2.6% 13% in small bowel, 80% in spleen (15,16,17,33)</td>
<td></td>
</tr>
<tr>
<td>Associated diseases</td>
<td>Ulcerating bowel disease, intussusception, body warts (22)</td>
<td>Carcinoma, sarcoma, benign neoplasms (16,17,38,39)</td>
<td>pancreatic carcinoma, lymphoid hyperplasia (1)</td>
</tr>
<tr>
<td>Morphology</td>
<td>Cleaved cell, immunoblastic, plasmacytoid (20)</td>
<td>Lymphocytic, epithelioid, plasmacytic, 'histiocytic', (16,17,38,39) rare leukemia (41)</td>
<td>Small lymphocytic MALATOMA, follicular lymphoma, large cell, anaplastic large cell, hairy cell leukemia, malignant histiocytosis, discordant lymphomas (present study)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate reference.
cytes accumulated large amounts of deleted extra-chromosomal viral genomes. Although virus particles were not detected by electron microscopy, as in our cases, high amounts of monomeric and oligomeric forms of extrachromosomal HaPV DNA molecules were found in the lymphoma cells (38,39).

Historically, two classes of hematologic lesions arising in SGH are distinguished on the basis of etiology. They are classified as either epizootic associated (20–23,35,37–39) or aging associated (16–18,40) lesions. We see major differences in onset, incidence, distribution, associated diseases and morphology from our finding (Table II). We describe a historically more complex lesion than seen in either the epizootic or aging-associated spontaneous lymphomas (Figure 2). Unlike the aging or epizootic lesions where splenic and bone marrow lesions such as myeloid leukemias (17,18,41) are rare, we describe a high incidence of splenic involvement and also several types of leukemia. Unlike the aging-associated lesions in SGH, we have detected splenic-marrow based lesions such as hairy cell leukemia and malignant histiocytosis in spleen.

Moreover, lesions previously unreported in epizootic or aging-associated lymphoma in SGH were observed, including anaplastic large cell lymphoma and follicular lymphoma. We found a frequency intermediate between epizootic and aging associated lesions. These findings suggest that we are describing a novel class of hematologic lesions in SGH which may be related to the induction of pancreatic carcinoma and diabetes in SGH.

One issue is why hematologic malignancies were not reported in previous BOP and/or STZ studies. This discrepancy may be due to a difference in dosing and administration regimens employed. While we used high doses of BOP and STZ and a sequential regimen of BOP followed by STZ, the previous reports by Pour et al. (11,12) of using combinations of BOP or STZ employed a different study protocol. In our study, (11) a single dose (20 mg/kg body weight) of BOP, or a single dose (50 mg/kg body weight) of STZ; followed at 14 days later with a single injection of the same dose of BOP (STZ + BOP group) were given to SGH. In another study, (12) STZ was used at 30 mg/kg alone; BOP, a single subcutaneous injection of 10 mg/kg alone and the STZ and BOP were injected simultaneously. Other studies also employed a different dosing regimen and utilized lower doses for BOP (10 mg/kg (43), 20 mg/kg (44)). In one study, (45) 12 weekly doses of 15 mg/kg of BOP were used and the SGH were sacrificed between 8–24 weeks, much earlier than the 40 week sacrifice schedule that we have followed in our SGH.

The above finding indicates a role for STZ, either directly or indirectly, in the development of hematologic lesions in SGH. The increased frequency and the unusual range of hematologic lesions along with a significant correlation between lymphoid hyperplasia and lymphoma in all groups, particularly the STZ-treated group, suggest a role of STZ in the development of these lesions. Whether these observations about STZ’s role in lymphomagenesis is a direct chemical effect or indirectly that of a co-carcinogen with a putative virus remains to be studied.

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