The cataractogenic effect of Dibromomannitol in rats

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Dibromomannitol (DBM) is one of the new chemotherapeutic compounds beneficial in the treatment of chronic myelogenous leukemia. Investigation of the effects of the drug on the rat lens has demonstrated that it exerts a complex cytotoxic action on the epithelial population and, with prolonged administration, is cataractogenic in normal animals. The response to DBM only partially resembles that evoked by Myleran, a related drug known to induce cataracts in rats.

Key words: drug-induced cataract, Dibromomannitol, lens epithelium cell, mitosis, DNA, biosynthesis, lens fibers, pharmacodynamics, histopathology, rats.

Dibromomannitol (1,6-dibromo-1,6-dideoxy-D-mannitol) is one of a series of polyalcohol derivatives with cytostatic effects on animal tumors. It shares with Myleran (1,4-dimethane sulfonoxbutane) the property of severely inhibiting myelopoiesis in normal animals without seriously affecting the lymphoid system. Clinical trials of dibromomannitol (DBM) have indicated that the compound is useful in the treatment of chronic myelogenous leukemia. It is considered to have fewer toxic side effects than Myleran, the agent most commonly employed in the therapy of this disease.

Prolonged administration of Myleran is known to induce cataracts in rats. The opacification appears to result from a profound inhibition of cell proliferation in the lens epithelium. Myleran-induced cataract in man has not as yet been reported, but recent observations suggest that opacities also may develop in the human lens after long term treatment.

In view of the similar therapeutic effect of Myleran and DBM, and the possible use of the newer drug in treatment of chronic myelogenous leukemia, we have examined its influence on the rat lens.

Materials and methods

Young male rats of the Osborne-Mendel strain (160 to 180 grams) were used. DBM was given to one group of animals as a single intraperitoneal injection of 1 Gm. per kilogram of body weight. The drug was administered as a fine dispersion in arachis oil. Rats were killed at intervals ranging from 8 hours to 4 weeks after injection. One hour prior to death, the treated animals and control rats of the same age received H\textsuperscript{3}-thymidine in a dose of 1 \textgreek{m}C per gram of body weight intraperitoneally. The specific activity of the tracer


compound was 3.0 C per millimole (Schwartz Bioresearch, Inc.). Autoradiographs of Feulgen-stained whole mounts of the lens epithelium were prepared, and the total number of mitoses and labeled nuclei were counted in each preparation at a magnification of 500×. Early prophase and late telophases were not included in the counts. Nuclei were considered labeled if there were 5 or more reduced silver grains over them. Average grain counts were calculated from the number of silver particles over 100 radioactive nuclei in one preparation from each animal.

To observe the effects of continuous administration of the drug, rats were fed a diet which contained DBM at levels of 1 or 2 Gm. per kilogram. Control animals were fed stock diet alone. Body weights and food consumption were recorded periodically and the eyes of all animals were examined with the slit lamp at weekly intervals. Rats of this group were killed after 1, 2, 3, 4, 8, 10, and 13 weeks of DBM feeding. In animals killed at 4 weeks or longer, one eye was processed for histologic examination and the other for the study of whole mounts of the lens epithelium.

### Results

**Effects of a single dose of DBM.** DBM-treated rats grew at a slightly reduced rate between the second and seventh day after injection of the drug but subsequently gained weight normally. Opacities of the lens or other outward signs of toxicity did not develop.

The drug affected cell proliferation in the lens epithelium within the first 24 hours (Table I, Fig. 1). Mitotic activity was reduced at 8 hours, and the number of dividing figures fell to a minimum of 60 per cent of normal values by 16 hours. At the end of the first day mitosis had returned to a normal level but a period

| Table I. Mitoses and H3-labeled cells in rat lens epithelium after a single dose of DBM* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Mitoses          | H3-labeled cells | Disintegrated nuclei, mean |
|                 | No. of          | Mean ± S.E.     | Per cent control | No. of          | Mean ± S.E.     | Per cent control |
|                 | preps.          |                  |                  | preps.          |                  |                  |
| 8 hours         |                 |                  |                  |                 |                  |                  |
| Control         | (4)             | 258 ± 12         |                  | (4)             | 2,326 ± 28       |                  |
| DBM             | (6)             | 180 ± 7          | 70               | (6)             | 2,475 ± 67       | 106              |
| 16 hours        |                 |                  |                  |                 |                  |                  |
| Control†        | (20)            | 210 ± 5          |                  | (20)            | 1,767 ± 64       |                  |
| DBM             | (6)             | 127 ± 9          | 60               | (4)             | 3,158 ± 247      | 179              |
| 24 hours        |                 |                  |                  |                 |                  |                  |
| DBM             | (10)            | 218 ± 12         | 104              | (10)            | 3,318 ± 189      | 188              |
| 2 days          |                 |                  |                  |                 |                  |                  |
| DBM             | (6)             | 245 ± 13         | 116              | (4)             | 3,429 ± 113      | 194              |
| 4 days          |                 |                  |                  |                 |                  |                  |
| DBM             | (4)             | 382 ± 31         | 182              | (4)             | 4,743 ± 620      | 268              |
| 7 days          |                 |                  |                  |                 |                  |                  |
| DBM             | (4)             | 438 ± 31         | 209              | (4)             | 2,740 ± 224      | 155              |
| 2 weeks         |                 |                  |                  |                 |                  |                  |
| Control         | (6)             | 209 ± 5          |                  | (6)             | 1,617 ± 68       |                  |
| DBM             | (6)             | 259 ± 10         | 123              | (6)             | 2,230 ± 30       | 138              |
| 4 weeks         |                 |                  |                  |                 |                  |                  |
| Control         | (5)             | 212 ± 9          |                  | (3)             | 1,637 ± 27       |                  |
| DBM             | (6)             | 249 ± 13         | 117              | (5)             | 1,415 ± 72       | 86               |

*One gram per kilogram intraperitoneally.
†The counts of all control animals killed between 16 hours and 1 week were grouped together to give a single average value as no significant differences were detected between specific intervals within this period. At 8 hours when animals were killed in the evening and at 2 and 4 weeks separate average counts were calculated for each interval.
of greater mitotic activity ensued, which lasted for at least 2 weeks. The relative incidence of prophase, metaphase, and telophase did not differ from control figures at any time interval. During the period of augmented mitotic activity, some abnormal dividing figures were noticed; approximately 10 per cent of the telophases had trailing or bridging chromosomes.

Clumps of Feulgen-positive material representing nuclear disintegration and cell death first appeared in appreciable quantities at 2 days, and increased in number at 4 days and 1 week. They were located predominantly in the germinative zone of the epithelium. Nuclear fragmentation was not found among the non-dividing differentiating cells of the meridional rows until 2 weeks after drug administration. Destruction of nuclei in the rows at this time was probably due to a latent injury sustained by the cells while they were in the germinative zone.

In contrast to the initial depression of mitotic activity after DBM injection, the number of cells labeled with H\textsuperscript{3}-thymidine rose during the first 24 hours. The counts at 8 hours did not differ from the controls, but by 16 hours there were nearly twice as many H\textsuperscript{3}-tagged cells in the treated animals. DNA-synthesizing cells reached a maximum level at 4 days and then fell gradually.

The early and rapid rise of H\textsuperscript{3}-labeled cells was associated with a reduction in the number of silver grains over the radioactive nuclei. A statistically significant difference (P<0.05) between the grain counts from control and treated rats could be demonstrated at 16, 24, and 48 hours after DBM injection. No difference was detected at later intervals.

The data indicate that DBM directly affected both DNA synthesis and mitosis in the lens epithelium. The apparent stimulation of proliferative activity following the initial decline of mitosis was accompanied by persisting evidence of cell death. The long-lasting effects of a single dose of the drug suggested that continuing treatment might injure the epithelial layer to an extent sufficient to induce cataract.

\textbf{Effect of chronic administration of DBM.}

Ten rats fed a diet containing DBM at a level of 1 gm. per kilogram consumed approximately 21 mg. of the drug per
Fig. 2. Effect of continuous DBM administration at 2 dose levels on mitosis and nuclear disintegration in the rat lens epithelium.

Day. This dose represents 90 to 95 mg. per kilogram of body weight daily. Two animals were killed at weekly intervals after the start of drug feeding until 4 weeks when the 4 surviving rats were killed. Weight gain of the treated rats was approximately 20 per cent less than that of control animals, but otherwise the experimental rats were considered healthy and in good condition. The lens remained clear during the observation period of one month.

The number of mitoses in the lens epithelium was almost twice the control value throughout the course of the experiment (Fig. 2). Remnants of fragmented nuclei were scattered in the germinative zone at one week. They increased in number later when they were found among the differentiating cells of the rows as well as in the areas of cell proliferation. At 4 weeks the germinative region displayed signs of a moderate cell loss. Some nuclei were double the normal size, and accessory micronuclei were abundant.

In view of the relatively mild damage of the epithelium after 4 weeks of feeding DBM at this level, a group of 18 rats was started on a diet containing 2 Gm. of DBM per kilogram that provided an average drug consumption of 150 to 190 mg. per kilogram of body weight daily. The higher dose inhibited growth by about 45 per cent, although the treatment otherwise seemed to be well tolerated. Six rats were killed within the first 3 weeks; they failed to show lens opacities. Cataracts did develop among the remaining animals.

The first change seen by slit lamp examination consisted of fine white dots in the most superficial layers of the cortex around the anterior pole. This lesion was seen at 2 weeks in the lenses of 4 of 16 rats, and by 4 weeks in 10 of the 12 surviving animals. With progression of the cataractous process a superficial net opacity or gray sheet extended throughout the anterior cortex. In these instances the pathologic changes in the anterior cortex prevented accurate examination of the lens nucleus and posterior cortex; however, dense posterior cataracts, visible even in gross examination, did develop in 2 animals.

During the first 4 weeks of drug feeding the apparent mitotic stimulation in the
lens epithelium resembled that in rats given the lower dose of DBM (Fig. 2), but nuclear destruction was more prominent as was the depopulation of the germinal zone and meridional rows. The 10 rats surviving for 8 weeks or longer demonstrated striking individual differences in the extent of the drug-induced injury, and for this reason the results will be discussed in order of severity of lens changes rather than duration of treatment.

Two animals failed to develop any lens opacities and were killed after 2 months. Mitotic activity in the lens epithelium was abnormally high and nuclear disintegration was frequent, but the integrity of the epithelial layer was well preserved despite some thinning of the population in the equatorial zone. Occasional swollen fibers were seen in sections of these lenses. The nuclei of the lens bow were normal although some were displaced posteriorly.

In 3 of the rats early diffuse anterior polar opacities were either nonprogressive or very slowly advancing. All of these animals showed marked depopulation of the lens epithelium including the meridional rows. Large areas were devoid of cells. Regardless of the decrease in the total number of cells in the epithelial layer, mitotic counts were equal to or higher than those of control animals. Fiber changes seen in histologic examination were more extensive than was suggested by the opacities visible in vivo. Swollen fibers were seen in the anterior and posterior cortex as well as in the equatorial region (Fig. 3). Caps interrupted the epithelial lining. The lens bow consisted of relatively few nuclei or could be identified only in rudimentary form.

Denser cataracts were observed in the last 5 animals of this group. The extensive changes in the anterior cortex were accompanied by posterior opacification in 3 eyes. When these rats were killed at 10 and 13 weeks, the entire equatorial area of the lens epithelium had disappeared (Fig. 4). Only the central part of the lens surface and portions of the pre-equatorial region were covered by a layer of cells. Mitotic counts were approximately 30 per cent of normal. A wide disparity in nuclear size characterized the surviving cells (Fig. 5). The nuclei varied from those with normal dimensions through several intermediate sizes to huge forms that resembled the polyploid cells induced

Fig. 3. Swollen anterior cortical fibers in a rat treated with DBM for 13 weeks. (Hematoxylin and eosin; phase; x300.)
A few giant mitotic figures were also seen in the DBM preparations indicating that the large nuclei were polyploid and that at least some of them were capable of mitotic division (Fig. 6). Superficial and deeper cortical fibers were swollen and filled with coarsely granular cytoplasm. In some regions, all the fiber structure was replaced by amorphous granular masses obviously resulting from further degeneration. The epithelial cells occasionally formed small multilayered heaps protruding into the superficial fiber layer. Swollen and misshapen cells with pyknotic nuclei replaced the lens bow. Large confluent cystic spaces occupied the equatorial region of one of the lenses with a dense posterior cataract (Fig. 7), while in the others a broad zone of fiber destruction completely surrounded the nucleus. Only a few islands of recognizable fibers were preserved.

Comment. Administration of a single dose of DBM interfered rapidly with both...
DNA synthesis and the mitotic process in the lens epithelium. The reduction in grain counts over labeled nuclei together with the increase in total number of labeled cells between 16 and 48 hours indicated that the rate of DNA synthesis was slowed. The initial suppression of mitosis apparent as early as 8 hours preceded the change in rate of H\textsuperscript{3}-thymidine incorporation and may have been caused by a direct action of DBM on the mitotic process. The secondary wave of increased proliferative activity possibly represented a response to the cell death which became manifest after 2 days, or it could have been due to a prolonged duration of the synthesiz-
Fig. 7. Large cystic spaces and extensive fiber damage in front of the equator after 10 weeks of DBM feeding. The epithelium ends in a multilayered formation. (Hematoxylin and eosin; phase; ×300.)

Continuous administration of DBM maintained mitotic counts at close to twice normal values during the first month. The response of the lens epithelium to more prolonged treatment was not uniform. In about half of the rats the cytopathologic process continued as in the earlier stages. Despite some reduction of the population of the germinative zone, the epithelial lining was intact. The remaining rats, in contrast, suffered almost total loss of the germinative area and the surviving cells were distinguished by a wide disparity in nuclear size.

The extent of lens fiber changes varied from animal to animal as did the epithelial injury. It was not possible to correlate the incipient dot opacities with a histologic counterpart, nor was all of the fiber damage seen in histologic examination identified in vivo. This applies particularly to lesions in the posterior cortex.

The process of cataract formation during chronic administration of DBM is similar to that of Myleran in some respects. Both agents act directly on cell proliferation in the epithelium and ultimately give rise to a profound depletion of the cell population. Abnormalities of the lens fibers appear a few weeks after cytopathologic changes in the epithelium are evident. The drug dose required for cataractogenesis, while high in comparison with therapeutic levels in man, is well tolerated by the animals and is below the lethal dose for both agents.

At the cellular level, however, the early effects of DBM on DNA synthesis and mitosis are not typical of Myleran but resemble the nitrogen mustard-like action of Triethylene melamine. This alkylating agent, as DBM, induces a transient slowdown of DNA synthesis and mitotic inhibition followed by a secondary wave of increased proliferative activity. Myleran, in contrast, blocks mitosis without altering the flow of cells through the synthesizing phase and leads to the accumulation of...
nuclei of increasingly higher ploidy and greater size. Evidence for this type of action by DBM was seen only after long-term treatment, when very large nuclei were common in the severely injured epithelial populations.

Elson and associates have described the hematologic response to DBM as a combination of nitrogen mustard and Myleran-type effects. Our results with the lens epithelium show a similar complex response. Recently, data have been presented indicating that the nitrogen mustard component of DBM action is due to the in vivo conversion of the substituted hexitol to 1,2:5,6-dianhydro-D-mannitol. The biologic effects of this diepoxide are similar to those of compounds of the nitrogen mustard group. The variation in response from animal to animal that we have noted during long-term DBM treatment could represent individual differences in the metabolic processing of the drug.

Posterior cortical opacities have been seen in patients with chronic myelogenous leukemia who had been treated for long periods of time with either Myleran or DBM. A possible role of the drugs in the production of these lens changes was suggested. Myleran is known to induce cataracts in rats, and in this study we have demonstrated the cataractogenic property of DBM. Under the conditions of the experiment the lens changes produced by DBM were more erratic in development and as a rule, less severe than those caused by Myleran. At the present time, no conclusions drawn from this investigation can be directly applied to the complex situation in patients treated with these 2 agents.

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REFERENCES

5. Institoris, L., Eckhardt, S., Horvath, I. P., and Sellei, C.: Comparative data on the action mechanism of 1,6-dibromo-1,6-dideoxy-D-mannitol and 1,4-dimethanesulfonyl-1,6-dideoxy-n-butane, Arzneimittelforschung. 16: 45, 1966.