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Effects of paraquat on development of preimplantation embryos in vivo and in vitro

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Abstract

Paraquat can cause oxidative stress through redox cycling, and preimplantation embryos are sensitive to oxidative stress in vitro. In this study, the effects of paraquat on preimplantation embryo development were examined. Exposure of preimplantation embryos (collected on the day after ovulation) to paraquat in vitro for 24 h at concentrations as low as 8 μM caused a significant decrease in the percentage of 8-cell embryos and an increase in the percentage of compacted morulae, but the content of reduced glutathione (GSH) in embryos was not changed. Altered embryo development was most likely due to premature compaction because a 42% decrease in cell number per compacted morulae was observed in embryos exposed to paraquat at 1 mM. Exposure of preimplantation embryos to paraquat in vitro for 4 days at 200 μM or higher eliminated development beyond the blastocyst stage. Exposure of bred female mice to paraquat at 30 mg/kg on day 2 after ovulation led to a small but significant decrease in the percentage of 8-cell embryos on day 3 without a detectable increase in the percentage of compacted morulae. No detectable change in preimplantation embryo development was found following paraquat exposure on the day of ovulation (day 0), although a significant decrease in embryo GSH was found on day 1. These data indicate that paraquat can adversely impact the development of preimplantation embryos in vitro and in vivo without consistent modulation of GSH level.
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1. Introduction

Reduced glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) is a storage and transport form of cysteine which also plays a major role in maintaining redox status within cells by protecting them from endogenous and xenobiotic reactive oxygen species and electrophiles [1]. GSH is synthesized de novo in a two-step process with the first step catalyzed by γ-glutamate-cysteine ligase (γ-GCL, the rate limiting enzyme) and with the second step catalyzed by glutathione synthetase.

The mammalian ovum and preimplantation embryo are dependent upon glutathione for normal development [2]. For example, appropriate levels of reduced glutathione (GSH) are required to permit decondensation of sperm chromatin after fertilization, a process that can be inhibited by lowering GSH levels in ova prior to fertilization [3–5]. Appropriate levels of GSH have also been associated with protection of some cells in the blastocyst: hydrogen peroxide in blastocoel fluid has been correlated with the apoptotic death of putatively redundant cells within the blastocyst, whereas cells in the inner cell mass, which ultimately forms the embryo, are protected by mechanisms involving GSH [6]. Glutathione has also been linked with thermotolerance in preimplantation embryos, as well as normal microtubule function and meiosis in oocytes [7–9].

Although appropriate levels of glutathione are required for development, the actual level of glutathione in embryos is not

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static during development. Nasr-Esfahani et al. [10] showed that glutathione levels drop through fertilization and development of mouse embryos by 45% to the 2-cell stage. Moreover, Gardiner and Reed [11] showed that GSH levels drop by 90% from the oocyte to the blastocyst stage. Mouse embryos developing through the 2-cell stage are likely dependent upon the glutathione stores that are cached prior to fertilization because 2-cell embryos, unlike blastocysts, are normally unable to synthesize GSH de novo [12]. However, under conditions of severe oxidative stress, such as following treatment with *tert*-butyl-hydroperoxide (*t*BH) which oxidizes cellular glutathione, 2-cell embryos can turn on the expression of γ-GCL and partially recover their GSH levels—under normal conditions, γ-GCL has not been detected prior to the blastocyst stage [13]. Importantly, recovery of GSH levels following oxidative stress in embryos can also be accomplished by reduction of oxidized glutathione (GSSG), an activity that is observed in both 2-cell and blastocyst stage embryos through the action of glutathione reductase [14].

Whereas some developmental stages of preimplantation embryos do have the capacity to regulate and recover their GSH levels in the face of oxidative stress, various studies have shown that the development of preimplantation embryos can still be significantly altered by exposure to chemicals that impact cellular redox status. Gardiner and Reed [11] showed that exposure of either 2-cell or blastocyst mouse embryos to *t*BH in vitro caused a reduction in development to later stages. Exposure of 2-cell mouse embryos to either diethylmaleate or L-buthionine *S*,*R*-sulfoximine in vitro (which depletes cellular GSH and inhibits γ-glutamate-cysteine ligase, respectively) also led to reduced development to later stages [12,14]. These in vitro studies suggest a marked sensitivity of preimplantation embryos to oxidative stress, but the relevance of this sensitivity to the in vivo situation is unclear. Indeed, Laub et al. [15] have shown that the embryo may be at least partially protected in vivo from chemicals that deplete GSH.

Paraquat (1,1′-dimethyl-4,4′-bipyridiniumdichloride; methylviologen; CASRN: 4685-14-7) is a broad-spectrum agricultural herbicide used in the United States and numerous other countries worldwide. Human exposures to paraquat may occur accidentally during manufacture or application, or they may occur intentionally as a method of suicide. Although less than 5% of a paraquat dose may be absorbed after oral exposure, absorption by the oral route is more efficient than following dermal or inhalation exposures, and, consequently, oral exposures often result in more severe toxicity [16–18].

Oxidative stress is a major mechanism for paraquat-induced cellular toxicity. Paraquat can be reduced by nicotinamide adenine dinucleotide phosphate (NADPH) and, in the presence of O_2, undergo redox cycling to form reactive oxygen species (ROS). Cellular dysfunction and damage may result from depletion of NADPH stores and the reaction of ROS with cellular molecules such as lipids and DNA [16,19]. Detoxification of some ROS can occur through the activity of superoxide dismutase and catalase. In addition, glutathione peroxidase (GPX) can use GSH to reduce the hydroperoxides that may form through the reaction of ROS with cellular molecules. GSSG is a byproduct of these reactions. Importantly, because GPX has a higher affinity for H_2O_2 than catalase does, GPX may play a major role in protecting against paraquat's toxicity [1]. However, the continual production of reactive oxygen species can lead to depletion of GSH and a decreased GSH:GSSG ratio indicating oxidative stress.

The most common toxicity of paraquat that has been reported in both humans and animals is that which occurs in the lung and is the leading cause of paraquat-induced mortality [16–21]. Sublethal long-term exposures to paraquat are the most common (e.g., occupational), and symptoms of pulmonary toxicity have been associated with these types of exposure as well [22,23]. Although teratogenicity is not generally observed following even high exposures to paraquat [18], a decrease in reproductive success in females has been suggested. One study in mice showed that continuous paraquat exposure in the diet at 125 mg/kg of feed, starting before pregnancy, resulted in a decrease in the number of litters produced without a reduction in litter size [24]. The mechanism responsible for those effects was investigated in this study. We hypothesized that exposure of bred female mice to paraquat would lower GSH levels leading to an inhibition of preimplantation embryo development.

2. Materials and methods

2.1. Animal husbandry and superovulation

Outbred non-Swiss albino mice [Hsd: NSA™ (CF-1®)] were produced in a breeding colony at the University of Northern Colorado from stocks originally obtained from Harlan (Indianapolis, IN). All animals were cared for in accordance with institutional guidelines as described previously [13]. Virgin or bred female mice were used in different experiments. Bred female mice were prepared as follows. Virgin pubertal female mice were synchronized and superovulated by i.p. injection of 10 IU equine chorionic gonadotrophin (eCG) followed 44–48 h later, and just prior to placing the females with proven breeder males, by i.p. injection with 5 IU of human chorionic gonadotrophin (hCG). On the following day (gestational day 0), the presence of copulation plugs was used as a positive selection criteria for animals to be used for further study.

2.2. Embryo collection and evaluation

Animals were euthanized using cervical dislocation. Embryos were collected by flushing the oviducts or uteri of bred female mice with M16 medium as previously described [13,26]. Collected embryos from individual mice were washed by successive passage through five 50-μL drops containing culture medium (M16 medium supplemented

with 4 mg/mL bovine serum albumen). Embryos were then evaluated by light microscopy for indication of fertilization, stage of development, quality of embryos, and abnormal features [26].

2.3. In vitro paraquat exposures

Embryos were isolated from synchronized, superovulated and bred female mice approximately 36 h after ovulation. Embryos were cultured (37 °C, humidified air plus 5% CO_2) under light paraffin oil in 10 μL drops of culture medium (10 embryos per drop) containing paraquat at 0, 8, 40, 200, or 1000 μM. After culturing, embryos were evaluated as above. Some cultures were examined at 24-h intervals for 4 consecutive days to determine the total percentage of embryos that successfully completed development to each of the various stages within that time frame. The number of cells per morula was determined in Hoechst 33258-stained embryos using fluorescent microscopy [27].

2.4. In vivo paraquat exposures

Mice were treated with saline (vehicle) or paraquat (30 mg/kg body weight, prepared fresh in saline) by i.p. injection of 100 μL per 10 g body weight. Treatments were given at approximately 12:00 h during the light cycle on the day of ovulation (day 0). Ovulation was estimated to have occurred at approximately 01:00 h during the previous dark cycle. The dose of paraquat used in these studies has been previously shown to significantly decrease hepatic glutathione concentrations in mice at 24 and 36 h post exposure [25], an effect that was confirmed in virgin female NSA mice in this laboratory (data not shown). Embryos were collected from bred female mice that were synchronized, superovulated and treated with paraquat as indicated in Fig. 1.

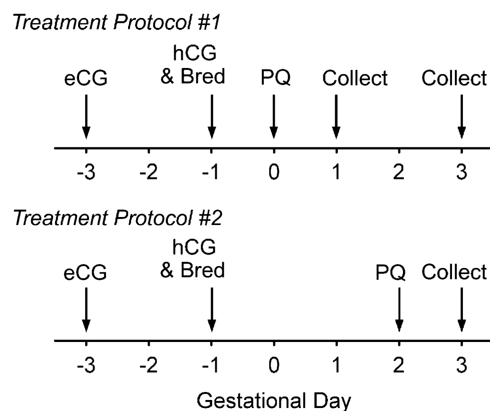

Fig. 1. Bred-female treatment regimens. Virgin pubertal female mice were synchronized and superovulated as described in Section 2. Animals were treated with either vehicle (saline) or paraquat (PQ, 30 mg/kg body weight) by i.p. injection when indicated for the different treatment protocols, and embryos were collected on the various days shown.

2.5. Evaluation of fetuses and gravid uteri

Normal bred pubertal female mice (neither synchronized nor superovulated) were euthanized on day 17 of gestation. Uteri were dissected and examined for resorptions and fetuses were evaluated for gross malformations as described previously [15].

2.6. GSH analysis

GSH content in embryos was determined using HPLC as described previously [15,28–30]. Pools of embryos were used (20–375) for analysis as required to permit detection. No degenerate embryos were analyzed.

2.7. Statistical analysis

Differences in morphological development were determined using arcsine transformation, analysis of variance, and Fisher's least significant differences procedures (LSD). For in vivo data, each sample represents embryos from a single dam. For in vitro data, each sample represents embryos within a culture drop. Differences in GSH and GSSG content were determined using analysis of variance and LSD. For in vivo data embryos from dams within each treatment group were pooled together and aliquoted into analysis pools which represent each sample. For in vitro data, embryos from culture drops within each treatment group were pooled together and aliquoted into analysis pools which represent each sample. Differences in mice maintaining pregnancy were determined using Kruskal-Wallis ANOVA by ranks analysis. Data are presented as means of all replicates within each treatment group across multiple trials ± standard errors. Means were considered significantly different if $p < 0.05$.

3. Results

The effect of paraquat on breeding outcomes was determined to verify the results of Dial and Dial [24]. Bred female mice were treated with saline (vehicle) or paraquat (30 mg/kg body weight) by i.p. injection on the day of ovulation. Seventeen days later (approximately full-term), dams and fetuses were examined for a variety of gross endpoints commonly associated with embryo toxicity [15]. As shown in Table 1, when compared to control mice, there were no significant differences in paraquat-treated mice for body, liver or uterine weight in dams, the number of fetuses per dam, the number of resorptions per dam, the total fetal weight per dam, individual fetal weight, or the number of fetal malformations. However, the percent of dams that were pregnant on day 17 was significantly reduced (24%) by paraquat exposure. A decrease in the number of pregnant dams without an increase in fetal resorptions suggested that paraquat exposure adversely impacted an event such as preimplantation development or very early post-implantation development.

Table 1
Effects of paraquat exposure on breeding outcomes

Measurement	Treatment	
	Saline	Paraquat
Number of females with copulation plugs	62	63
Dam weight on day 17 (g)	51.7 ± 0.8	50.0 ± 1.1
Dam liver weight (g)	2.8 ± 0.1	2.7 ± 0.1
Dam uterine weight (g)	16.7 ± 0.7	15.8 ± 0.8
Number of fetuses/dam	12.0 ± 0.6	11.4 ± 0.7
Number of resorptions/dam	2.0 ± 0.4	2.1 ± 0.4
Fetal weight (g)	0.91 ± 0.01	0.91 ± 0.01
Number of fetal malformations	0	0
Total fetal weight per dam (g)	11.1 ± .5	10.3 ± 0.6
Percent of dams pregnant on day 17 (full-term)[a]	93.1 ± 4.4%	70.6 ± 10.2*

Note: Bred dams were injected (i.p.) with saline or paraquat (30 mg/kg) on the day of ovulation (d0). Data are presented as mean ± S.E.

[a] The percent of dams maintaining pregnancy was calculated by taking the number of pregnant dams on day 17 divided by the total number of dams with copulation plugs on day 0 multiplied by 100.

* Indicates that the mean is significantly different from control ($p < 0.05$).

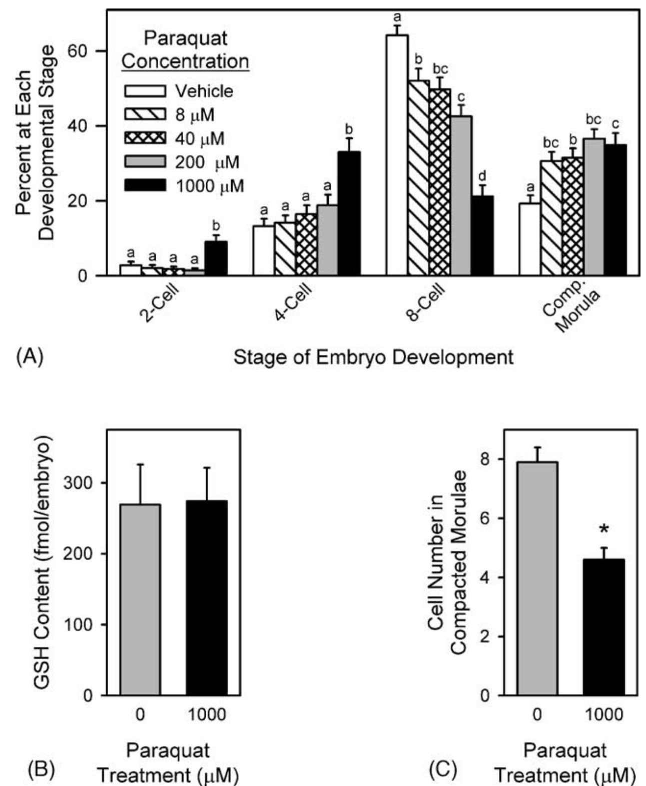

Fig. 2. Paraquat altered the developmental stage profile of early preimplantation embryos in vitro. Embryos were collected from bred female mice one day after ovulation and cultured in medium supplemented with paraquat for 24 h. (A) Developmental stages were determined by microscopy ($N \geq 13$ embryo pools per treatment group): 2-cell, 4-cell, 8-cell, and compacted morula. Degenerate and blastocyst embryos comprised 1% or less of the total embryos recovered in any treatment group (data not shown). (B) Non-degenerate embryos were isolated and pooled (within groups as shown, $N \geq 13$ pools per treatment group) for analysis of GSH content. (C) Cell number within compacted morulae was determined by fluoresence microscopy ($N \geq 28$ embryos per treatment group). Data are presented as mean ± S.E. Different letters indicate a significant difference between means within each developmental stage. *Indicates a significant difference in cell number.

In vitro studies were used to examine the impact of paraquat exposure on the development of preimplantation embryos. Embryos were isolated on day 1 from bred, superovulated female mice and non-degenerate embryos were cultured in the presence or absence of paraquat at concentrations from 0 to 1000 μM for 24 h. Exposure of embryos to paraquat at 1000 μM significantly increased the percentage of embryos in both the 2-cell and 4-cell stages (Fig. 2A). In contrast, a dose-dependent decrease in the percentage of 8-cell embryos was observed by as much as 67% at the 1000 μM paraquat concentration, and with a concentration as low as 8 μM being sufficient to significantly decrease the percent of 8-cell embryos (Fig. 2A). The opposite effect was seen in compacted morulae, which were dose-dependently increased in percentage by as much as 81% at the 1000 μM paraquat concentration (Fig. 2A). When considered together, these data suggested an inhibition of development to the 8-cell stage and an acceleration of embryo compaction. Interestingly, there was no significant difference in GSH content between embryos exposed to paraquat at 0 or 1000 μM (Fig. 2B).

To examine the impact of paraquat exposure on embryo compaction, embryos were isolated on day 1 from bred, superovulated female mice and non-degenerate embryos were cultured with 0 or 1000 μM paraquat. After 24 h, compacted morulae were isolated and examined for cell number by fluorescence microscopy. As shown in Fig. 2C, the average number of cells in compacted morulae was significantly decreased by 42% from 7.9 in control cultures to 4.6 in paraquat cultures. These data support the suggestion that paraquat triggers premature compaction.

To examine the overall impact of paraquat exposure in vitro on preimplantation development, embryos were isolated on day 1 from bred, superovulated female mice and non-degenerate embryos were cultured in the presence or absence of paraquat at concentrations from 0 to 1000 μM for 4 days, or to approximately day 5 post ovulation. After 1, 2, 3, and 4 days of culture, the embryos were evaluated for developmental stage to determine the percentage of total embryos that had developed to each developmental stage. As shown in Fig. 3, paraquat significantly decreased the successful development of preimplantation embryos to the 8-cell stage and beyond. A dose-dependent effect of paraquat on development was apparent such that, relative to control cultures, the percentages of embryos successfully developing to later stages decreased as the paraquat concentration increased. At the lowest paraquat concentration tested (8 μM), development to the hatching blastocyst stage was reduced by approximately 15%, whereas 40 μM paraquat reduced development to that stage by approximately 60%. At a 200 μM paraquat concentration, development beyond the blastocyst stage was eliminated, and, at 1000 μM, development beyond the earlier compacted morula stage was eliminated.

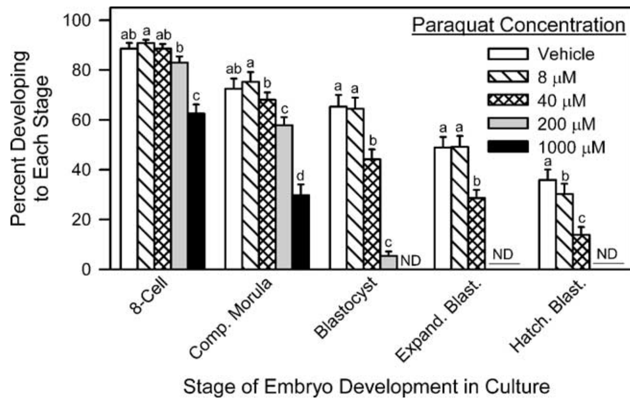

Fig. 3. Paraquat inhibited embryo development throughout the preimplantation period. Embryos were collected from bred, superovulated female mice 1 day after ovulation and cultured in medium supplemented with paraquat at a set concentration for 4 days. The percentage of embryos at each stage of development (8-cell, compacted morula, blastocyst, expanded blastocyst, or hatching blastocyst) was determined at 24-h intervals for each treatment group. Data are presented as the percentage of total embryos (±S.E.) that had developed to the given developmental stage by day five post ovulation ($N \geq 22$ embryo pools per treatment group). Different letters indicate a significant difference between means within each developmental stage. ND, none detected.

To determine if preimplantation embryos are sensitive to paraquat-induced toxicity in vivo, embryos were isolated on day 1 from bred, superovulated female mice that were treated with saline or paraquat (30 mg/kg) on the day of ovulation (Treatment Protocol #1, Fig. 1). Non-degenerate embryos in both saline- and paraquat-treated mice were found to be predominantly at the 2-cell stage when isolated on day 1 (Fig. 4A), and there was no significant difference between the percentages of any developmental stages between the two treatment groups. However, the GSH content of non-degenerate embryos on day 1 was significantly reduced (by

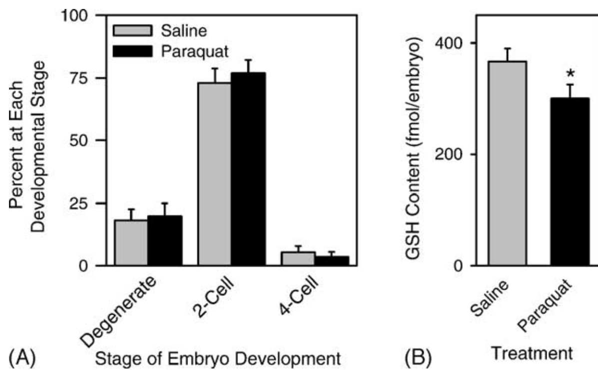

Fig. 4. Paraquat exposure on the day of ovulation (day 0) decreased embryo GSH content but did not alter embryo development at day 1. Bred female mice were injected with saline or paraquat (30 mg/kg body weight) at approximately 12:00 h after ovulation. Twenty-four hours later (A), dams were killed and embryos were collected for assessment of developmental stage by microscopy ($N \geq 26$ mice per treatment group). Non-degenerate embryos were pooled ($N \geq 24$ embryo pools per treatment group) for analysis of GSH content (B). Data are presented as mean ± S.E. *Indicates that the mean was significantly different from control.

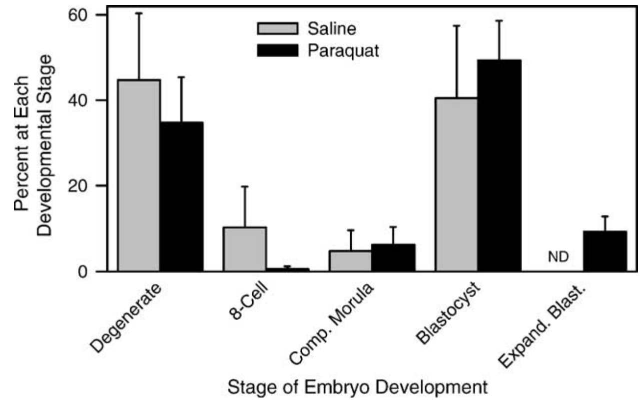

Fig. 5. Paraquat exposure on the day of ovulation (day 0) did not significantly alter embryo developmental day 3. Bred female mice were exposed to paraquat as in Fig. 2. Three days later, dams were killed and embryos were collected for assessment of developmental stage by microscopy: degenerate, 8-cell, compacted morula, blastocyst, or expanded blastocyst. Data are presented as mean ± S.E. ($N \geq 9$ mice per treatment group). ND, none detected.

18%) in paraquat-treated mice relative to embryos from control mice (Fig. 4B). These data suggest that paraquat exposure in vivo may induce oxidative stress in 2-cell embryos, leading to a significant reduction in GSH content but no significant change in development.

To determine if paraquat exposure could alter the development of embryos beyond the 2-cell stage, embryos were isolated on day 3 from bred, superovulated female mice that were treated with saline or paraquat (30 mg/kg) on 1 of 2 different days: day 0 or day 2. After treatment on day 0 (the day of ovulation—Treatment Protocol #1, Fig. 1), non-degenerate embryos were found to be predominantly at the blastocyst stage, and there were no significant differences in the percentages of any developmental stage between saline- and paraquat-treated mice, nor was there a difference in the percentage of degenerate embryos (Fig. 5). Nevertheless, paraquat treatment seemed to cause a slight (but not significant) decrease in the percentage of 8-cell embryos (Fig. 5). After treatment on day 2 (Treatment Protocol #2, Fig. 1), non-degenerate embryos were again found to be predominantly at the blastocyst stage, and a decrease in the percentage of 8-cell embryos was seen in paraquat-treated mice that was statistically significant (Fig. 6A). A slight (but not significant) increase in the percentage of compacted morulae was also observed (Fig. 6A). These data suggested that development up to or through the 8-cell stage may be impacted by in vivo exposure to paraquat. GSH levels in day 3 embryos were also examined at 24 h after paraquat treatment on day 2. Unlike for day 1 embryos, paraquat did not significantly alter embryo GSH content on day 3 (Fig. 6B).

4. Discussion

In a previous study by Dial and Dial [24], paraquat was shown to significantly decrease the number of litters pro-

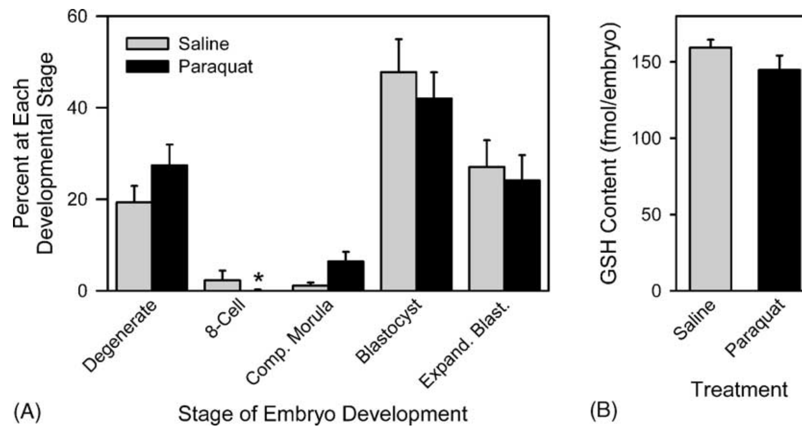

Fig. 6. Paraquat exposure on day 2 decreased embryo development on day 3 but failed to decrease embryo GSH. Bred female mice were injected with paraquat (i.p., 0 or 30 mg/kg body weight) at approximately 12:00 h 2 days after ovulation. Twenty-four hours later, animals were killed for assessment of embryo developmental stage ($N \geq 23$ mice per treatment group) (A) as in Fig. 2, and non-degenerate embryos were pooled ($N \geq 13$ embryo pools per treatment group) for analysis of GSH content (B). Data are presented as mean ± S.E. *Indicates that the mean was significantly different from control.

duced by breeding pairs by 21% without inducing significant maternal toxicity. A curious additional finding of their study was a lack of any paraquat effect on litter size, which they suggested indicated a lack of significant fetal resorption. Because the exposure to paraquat was continuous, it was also unclear what exposure time frame was key to mediating the reduction in litter numbers. An earlier study by Bus et al. [31] examined the effects of mid-gestational paraquat exposure in pregnant mice and found no significant changes in resorption rates at doses below those that caused maternal mortality, and, importantly, they did not report a decrease in successful litters. In contrast, as shown here (Table 1), paraquat exposure on the day of ovulation caused a 24% decrease in litter number. Combined, these results suggested early gestation as the sensitive time frame for paraquat exposure to reduce litter number. Ovulation would not have been impacted because all paraquat exposures occurred after this event.

The results of the studies presented here strongly suggest that paraquat induces preimplantation embryos to undergo premature compaction in vitro, a process that probably begins after reaching the 4-cell stage (Fig. 2) and then precipitates a failure of development to later stages (Fig. 3). An early rather than late developmental stage target for paraquat is supported by O'Fallon and Wright [32] who studied the effects of paraquat on metabolic pathways in mouse morulae and expanded blastocysts at concentrations of 1000 μM and higher and found no overt paraquat toxicity. In contrast to our original hypothesis, the mechanism underlying paraquat-induced premature compaction may not involve glutathione directly. Twenty-four hours of in vitro paraquat exposure at the highest concentration (1000 μM) failed to significantly alter GSH levels in preimplantation embryos, although marked changes in development were observed (Fig. 2). A failure to detect a decrease in GSH levels in preimplantation embryos in this study may have been a consequence of the chosen sampling time point—a different time point may have exhibited decreased GSH and increased GSSG. Alternatively, the failure to detect decreased GSH levels may be because preimplantation embryos have the ability to reduce GSSG to GSH as early as the 2-cell stage [14]. Moreover, under conditions of severe oxidative stress, 2-cell embryos can turn on the expression of γ-GCL to synthesize GSH de novo [13]. Due to the limits of sensitivity for the methods used and the small amount of material that can be obtained from preimplantation embryos, attempts to measure GSSG levels in embryos were unsuccessful. Regardless of the lack of a significant in vitro change in GSH levels in this study, previous studies of preimplantation embryos, in which depletion of GSH was accomplished using chemicals other than paraquat, failed to induce premature compaction [11,12,14,15,28], results that support the suggestion that premature compaction may not involve glutathione directly.

A dose of paraquat that significantly reduced litter number (30 mg/kg body weight, Table 1) had a minimal effect on preimplantation development in vivo (Figs. 4–6). Indeed, the only significant effect was a decrease in 8-cell embryos, and this was only observed when paraquat was given on day 2 after ovulation (rather than day 0), a time point when development through the 8-cell stage was expected to be occurring (Fig. 2A and [26]). These results support our in vitro results and suggest that the developmental period from the 4-cell to the 8-cell embryo is the most sensitive window for exposure to paraquat. The elimination half-life of paraquat has been estimated to be approximately 40 h in rats and 80 h in humans [33,34]. Therefore, it is likely that paraquat concentrations in the reproductive tracts of pregnant mice drop markedly over time, unlike in culture, a pharmacokinetic consideration that could account for the lack of any significant effect when paraquat was given earlier than day 2.

In vivo paraquat exposure on the day of ovulation (day 0) led to a significant decrease in GSH content in embryos on day 1 as expected because 2-cell embryos are normally unable to synthesize GSH de novo (Fig. 4B) [12]. The decrease in GSH levels suggest that 2-cell embryos responded

to paraquat as liver does ([25] and Hausburg, unpublished results), and oxidative stress may have occurred. It is not clear what the fate of GSH was in paraquat-exposed embryos, but it is likely to have formed mixed disulfides and GSSG. Attempts to measure GSSG levels in day 1 embryos were unsuccessful. Gardiner and Reed [11] showed that exposure of 2-cell mouse embryos to *t*BH (13 μM) in vitro for 15 min led to a greater than 80% decrease in cellular GSH content which could be entirely accounted for by increased levels of GSSG and protein mixed-disulfides with the latter being the greatest component of the oxidized glutathione. When GSH levels in day 3 embryos were examined after in vivo paraquat exposure on day 2, they were found to be not significantly different from controls (Fig. 6B). This was expected because the predominant developmental stage on day 3 was the blastocyst, and mouse blastocysts are known to be able to both synthesize GSH de novo and reduce GSSG through the activity of glutathione reductase [12,14].

The experimental results presented here indicate that paraquat exposure can profoundly alter the development of preimplantation embryos in vitro leading to a failure of developmental progression at some paraquat concentrations. A likely cause of the failed developmental progression is enhanced and premature embryo compaction. Some evidence for a similar form of embryo toxicity was observed in vivo. However, it is difficult to reconcile the limited evidence of in vivo embryo toxicity with a significant decrease in the number of dams maintaining pregnancy even though both observations were made in animals that received the same dose of paraquat. Future studies will address this question.

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