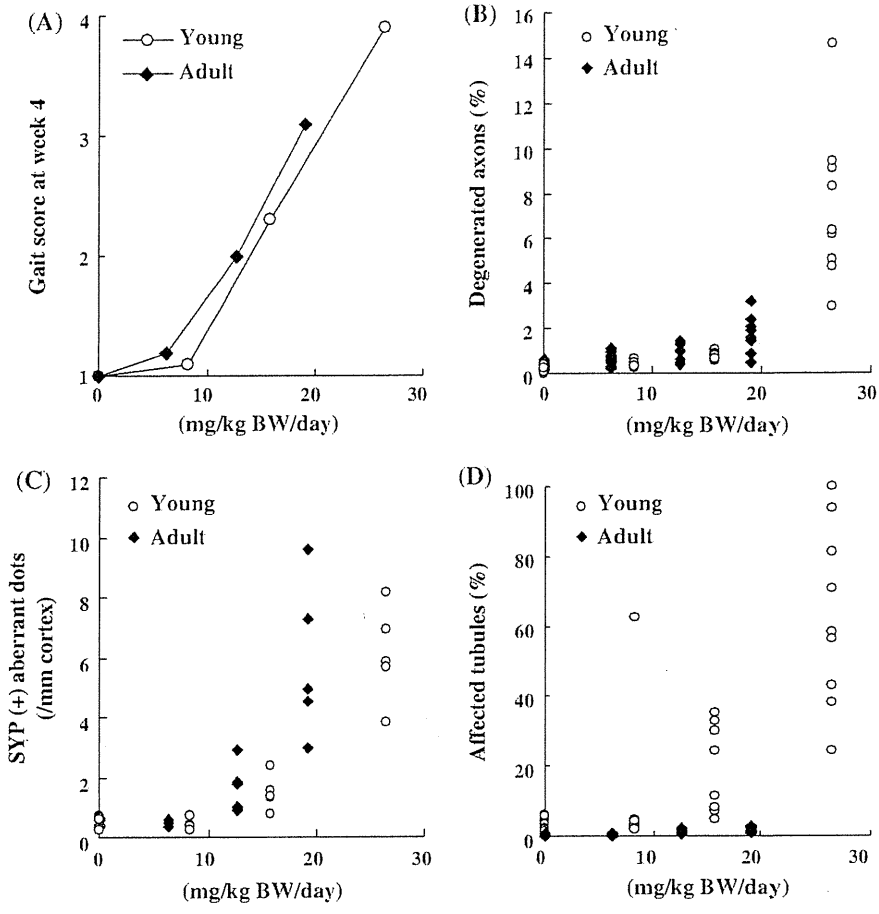

Fig. 6 Relationship between ACR intake per body weight and changes in neurotoxicity and testicular toxicity parameters in young and adult rats

here observed in the young group. Also, neurotoxic lesions such as central chromatolysis of ganglion cells in the trigeminal nerves, degenerated axons in the sciatic nerve and dot-like SYP-immunoreactive structures in the cerebellar molecular layer, were evident from 100 ppm in both young and adult groups. The magnitude of changes in these parameters was higher in the young group than in the adult group, especially at the highest dose, and neurotoxicity appeared stronger in young animals, though the types of lesions observed were similar between the young and adult groups. Compared to adult animals, intake of ACR per kg body weight was higher in young animals at each dose and the parameters indicating the neurotoxicity increased in proportion to ACR intake. Accordingly, the stronger neurotoxicity in the young animals can be considered to be a reflection of larger amount of ACR intake per body weight. These results suggest that the susceptibility to ACR-induced neurotoxicity in young and adult rats is qualitatively similar under the given experimental conditions. As mentioned in the Introduction section, a few studies have demonstrated life stage-related differences in susceptibility to ACR neurotoxicity, though the experimental conditions, such as age of animals, dosing methods, and parameters examined, were different. While Suzuki and Pfaff concluded that suckling rats were more susceptible (Suzuki and Pfaff 1973), it seems that there was not much difference in number of injections to cause apparent symptoms and myelin degeneration between suckling and adult rats. In the report by Ko et al., earlier occurrence and faster progression of neurological abnormalities in young animals were similar to those observed in our study (Ko et al. 1999). Although the authors stated that the daily intake was not significantly different between the young and adult groups, intake of ACR per body weight at the beginning of the experiment might have been higher in the young group, because younger animals usually take more water than older ones. Taken together, clear evidence of the susceptibility difference in neurotoxicity between young and adults animals is considered to be undetermined.

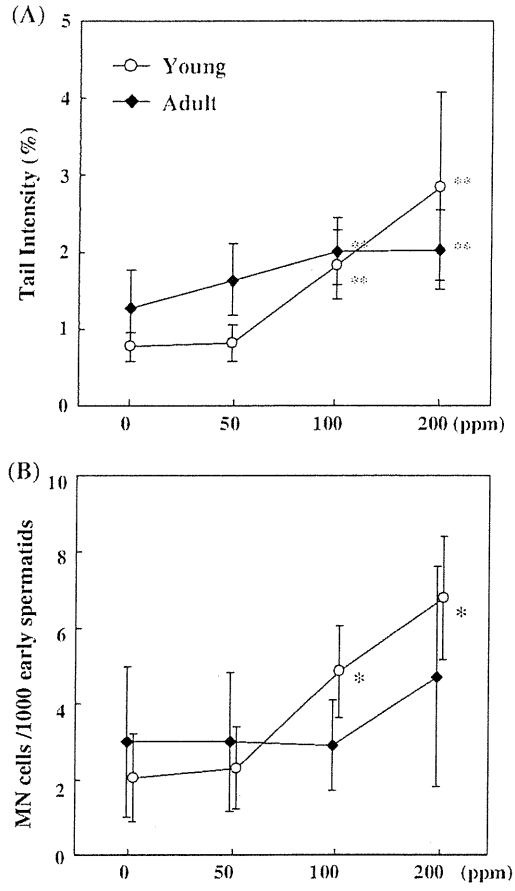

Fig. 7 Tail intensity of the comet image (**a**) and micronuclei frequency (**b**) obtained from young and adult rats given ACR in the drinking water for 4 weeks. Data are mean ± SD. $^{**}P < 0.01$ vs. 0 ppm

Regarding the susceptibility to ACR testicular toxicity in the present study, young animals showed apparently diverse and more profound lesions exceeding the dose–effect relationship observed in adult animals. ACR is known to interfere with motor proteins such as kinesin found in the sperm flagellum and alkylate protein sulfhydryl groups in the sperm tail (Sickles et al. 2007; Friedman et al. 2008). Therefore, it is considered that elongate spermatids are highly susceptible to ACR. In the comet assay, although DNA damage in the young group was higher than that in the adult group at 200 ppm, the values were not greatly different. However, the MN test revealed that ACR clearly induced MN in the young group, but not in the adult group. These results well correspond with the observations on histopathological examination. Because the comet assay and MN test in the testis target spermatocytes and early spermatids, the late stage of spermatogenesis may be more susceptible to ACR-induced genotoxicity in young than in adult animals.

As reported by others (Yousef and El-Demerdash 2006), the basal level of testicular GST activity in our cases was much lower than that in the liver. Although there were no life stage differences in the liver levels of GST activity, testicular GST activity in the present study was significantly lower in the young groups, irrespective of the ACR treatment. The activity of GST is low at birth and then increases gradually, but it has been known that the developmental profiles of antioxidant enzymes including GST in the testis differ greatly from those in the liver (Peltola et al. 1992). A study of the immunolocalization of GST-Yo, a member of the mu class expressed at high levels in the testis and epididymides, revealed that this enzyme was not detectable until 39 days of age and then appeared mainly in the elongate spermatids, with expression reaching maturity by day 49 (Papp et al. 1994). Therefore, the detoxification capacity of the testis in young animals was considered to be much lower than that in the adult animals during the experimental period in the present study, and such a difference might reasonably account for the high susceptibility to ACR-induced testicular toxicity observed in our young animals. In the liver, although GST activity was increased at 200 ppm, there were no apparent life-stage differences. Considering that the liver is the main organ involved in detoxification of ACR, similar level of GST activity may have contributed to the lack of differences in susceptibility to neurotoxicity between young and adult rats. Increase in GST activity in ACR-treated rats has been reported and considered to be due to increased formation of S-conjugates between ACR and GSH (Yousef and El-Demerdash 2006). ACR is known to cause GSH depletion (Zhang et al. 2009); however, decrease in GSH contents was not found in the present study. Because recovery or rather increase in liver GSH contents after depletion by treatment animals with acetaminophen has been reported (Ishii et al. 2009), the level of GSH in the present study might possibly have recovered after repeated treatment with ACR during the experimental period.

In summary, our results suggest that susceptibility to ACR neurotoxicity in young animals might not be different from that in adult ones when exposure levels are adjusted for the body weight. Regarding testicular toxicity, young animals proved more vulnerable than adults, and this might be due to a low level of testicular GST activity.

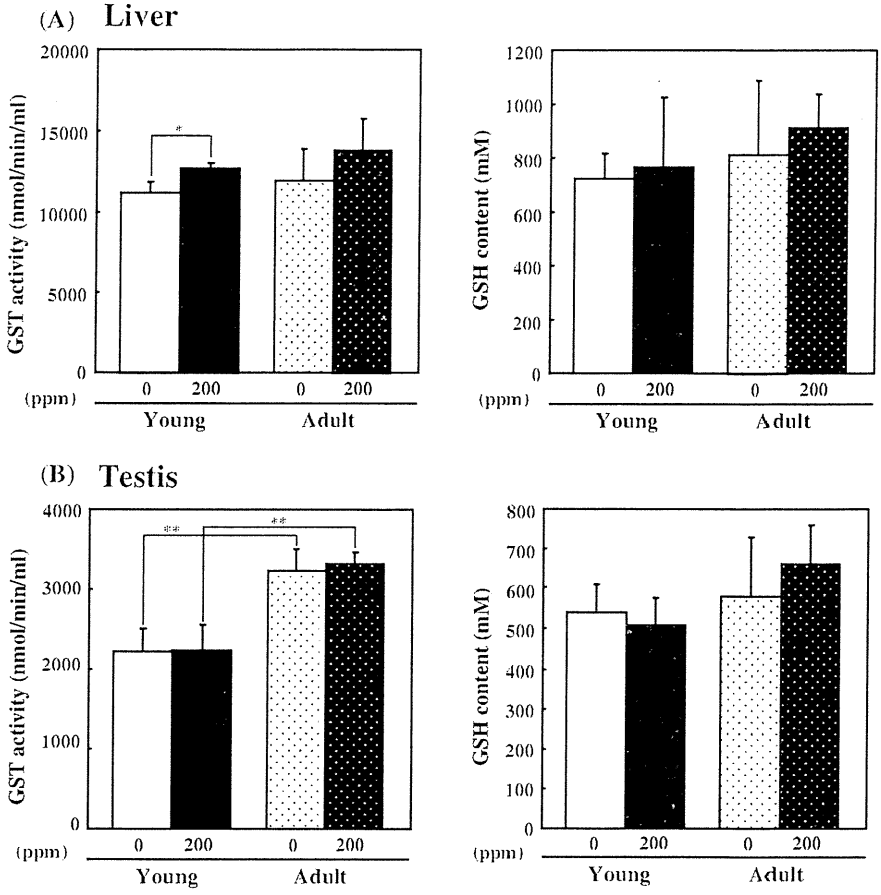

Fig. 8 GST activity and GSH contents in the liver (**a**) and testis (**b**) of young and adult rats given ACR at 0 or 200 ppm for 4 weeks. Data are mean ± SD. *, **$P < 0.05$ and $P < 0.01$

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