Sex-dependent action of prenatal fractionated X-irradiation in the mouse. Biochemical findings

L. Weber and W. Schmahl[1]

Abteilung Zellchemie und Abteilung Nuklearbiologie der Gesellschaft für Strahlen- und Umweltforschung GSF München, D-8042 Neuherberg (Federal Republic of Germany), 26 July 1979

Summary. X-Irradiation of pregnant NMRI-mice on gestational days 11-13 with 3 × 1.05 Gy increased postnatal mortality of the female offspring only. Weights, protein content and acetylcholinesterase, as well as Na,K-ATPase activities in the brains of all treated offspring, were changed. There were, however, no differences between females and males with respect to these parameters.

Examining the action of fractionated X-irradiation on the mouse fetus, Schmahl et al. reported that the particular dose of 3 × 1.05 Gy, but not 3 × 0.95 or 3 × 1.14 Gy, given on gestational days 11-13, caused a remarkably higher mortality of the female offspring within 48 h after birth[2]. The evident symptoms were undercooling and starvation, the latter produced by an inability, primarily of newborn females, to search for their mothers' nipples[2].

Sex differentiation in the mouse takes place only after the time at which the radiation was applied[3]. Such a radiation dose will suppress any sexual differentiation[4]. Schmahl et al. found a) a higher number of rosettes in the brains of female mice compared with the brains of males after this X-irradiation[2], and b) more severe lesions of the thalamic regions of these females' brains[5]. The center of thermoregulation in mammals is localized in the thalamus[6]. Suckling in rat pups is caused by olfactory stimuli[7], and these stimuli are processed in the thalamus[8]. Therefore, the origin of the findings of the present and preceding studies[2,5] had to be localized in the brain.

We have now investigated the postnatal brain weights, their protein contents and the activities of two enzymes, acetylcholinesterase and Na,K-ATPase, in the brains of mice prenatally X-irradiated with 3 × 1.05 Gy, with special respect to differences between females and males. In a previous communication we showed that a single X-ray dose given to female mice on the 12th day of gestation caused postnatal changes in the activities of these enzymes in the brains of the offspring[9].

Material and methods. NMRI-mice of the Neuherberg strain were used. The housing, mating and radiation treatment conditions have been described previously[10]. Acetylcholinesterase activities in the brains were determined according to Ellman et al.[11], those of Na,K-ATPase according to Post and Sen[12]. The protein content of the wet brain tissue was determined with a laboratory modification of the Lowry method[13]. The details of these procedures have been given in a previous paper[9]. Enzyme activities were expressed as milliunits per mg of protein, 1 enzyme unit being defined as the formation of 1 µmole of product per min at 37 °C. Statistical analyses were performed with the t-test at a significance level $a \leq 0.05$. Brain weights, protein contents and the specific activities of the enzymes of control females and males, as well as of prenatally X-irradiated females and males, were compared for each of the postnatal days 0 (= day of birth), 2, 8, 12, 23, 34, and 64, with a total of 12 controls and 8 irradiated animals (day 0: n = 12) per day.

Results. The fractionated X-irradiation as described in the method section resulted in the data presented in the table. The following differences between controls and treated animals were obtained: a) Brain weights of both X-irradiated females and males were decreased by about one half throughout the investigation period. b) The protein content of the brains of the treated offspring was altered, and values above and below controls were found, most values differing significantly. c) Brain acetylcholinesterase activities of the treated offspring exceeded those of the controls on all days examined, but the differences were less significant. d) Na,K-ATPase activities were changed slightly, but with hardly any significant differences. e) Control females and males showed no sex differences in their brain weights, protein contents, acetylcholinesterase and Na,K-ATPase activities. f) Brain weights, protein contents and the 2 enzyme activities of the animals which were prenatally X-irradiated with 3 × 1.05 Gy showed likewise no sex differences at all.

Discussion. In a previous study we showed that a single X-ray dose (1.90, 0.95 or 0.48 Gy on the 12th gestational day) given to pregnant mice altered postnatal brain enzyme

Mean values of weights, protein contents and 2 enzyme activities in the brains of NMRI-mice. C = controls, X = animals treated with 3 × 1.05 Gy on gestational days 11-13. ◐ = significant difference, $a \leq 0.05$. Each day's group comprized 2-9 animals of either sex with a total of 12 animals (C) and 8 animals (X) per day. Standard deviations have been omitted to avoid confusion; they ranged from 2 to 10% (weights), 3 to 7% (protein contents), 1 to 11% (acetylcholinesterase), and 3 to 17% (Na,K-ATPase) of the respective mean values

Postnatal age (days)			0	2	8	12	23	34	64
Brain weights (mg)	♂	C	93	139 ◐	258 ◐	364 ◐	403 ◐	331 ◐	469 ◐
		X	41 ◐	49 ◐	133 ◐	191 ◐	209	190 ◐	228 ◐
	♀	X	40 ◐	46 ◐	121 ◐	192 ◐	195 ◐	200 ◐	226 ◐
		C	90 ◐	134 ◐	254 ◐	364 ◐	407	439 ◐	475 ◐
Protein contents (%)	♂	C	7.7	7.4 ◐	7.4 ◐	10.3 ◐	11.9 ◐	13.3	12.5 ◐
		X	7.4	8.1 ◐	7.0 ◐	7.8 ◐	10.4 ◐	12.4	11.6 ◐
	♀	X	7.4	8.2 ◐	7.1 ◐	7.3 ◐	10.9 ◐	12.8	11.9
		C	7.8	7.5 ◐	7.4 ◐	9.4 ◐	12.0 ◐	12.9	12.5
Acetylcholinesterase (mU/mg)	♂	C	40	47 ◐	80 ◐	125 ◐	165 ◐	172 ◐	185 ◐
		X	47	50 ◐	112 ◐	143 ◐	193 ◐	206 ◐	228 ◐
	♀	X	44	46 ◐	113 ◐	141 ◐	190 ◐	185	215 ◐
		C	40	49 ◐	80 ◐	128 ◐	167 ◐	176	189 ◐
Na, K-ATPase (mU/mg)	♂	C	41 ◐	58	126	157	254	258	275
		X	49 ◐	53	104	143	246	244	301
	♀	X	48	48 ◐	116	153 ◐	240	229	303
		C	42	59 ◐	124	174 ◐	235	264	294

activities of the offspring[9]. Similar findings have been reported by others[14]. X-irradiation of pregnant mice on gestational days 11, 12 and 13, with 1.05 Gy each time, also caused marked and significant changes in postnatal brain weights, protein content, acetylcholinesterase and Na,K-ATPase activities of all offspring (table). Increased mortality, however, was observed only with females and within 48 h after birth[2]. The enzyme activities and protein contents of the brains of irradiated females did not, even on the day of birth and the 2nd postnatal day, differ from those of the corresponding males.

Thus, these biochemical data do not help to explain the conspicuous mortality of the female offspring caused by this particular X-ray dose. It may be concluded that the investigation of postnatal brain acetylcholinesterase and Na,K-ATPase activities is suitable for the detection of more general radiation damage, but not the specialized damage being studied in this and previous investigations[2,5]. At present we are examining X-chromosome-linked enzyme activities in the brains of mice prenatally X-irradiated with 3×1.05 Gy.

1 We gratefully acknowledge the assistance of Miss C. Gutmann.

2 W. Schmahl, E. Senft, L. Weber and H. Kriegel, Experientia *35*, 541 (1979).
3 K. Theiler, in: The house mouse, p. 101. Springer Verlag, Berlin, Heidelberg, New York 1972.
4 D.J. Nash, Biol. Bull. *137*, 189 (1969).
5 W. Schmahl, L. Weber and H. Kriegel, Poster presented at 6th Eur. Teratology Society Conference, Budapest-Szentendre (Hungary) 1978.
6 U. Söderberg and K. Larson, Physiol. Behav. *17*, 993 (1976).
7 M.H. Teicher and E.M. Blass, Science *198*, 635 (1977).
8 E. Eitschberger, Adv. Anat. Embryol. Cell Biol. *42*, 64 (1970).
9 L. Weber, W. Schmahl and H. Kriegel, Strahlentherapie *155*, 425 (1979).
10 W. Schmahl, L. Weber and H. Kriegel, Strahlentherapie *155*, 347 (1979).
11 G.L. Ellman, K.D. Courtney, V. Andres, Jr, and R.M. Featherstone, Biochem. Pharmac. *7*, 88 (1961).
12 R.L. Post and A.K. Sen, Meth. Enzymol. *10*, 762 (1967).
13 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
14 K. Ueda, M. Ude and Y. Yoshizawa: J. Radiat. Res. *15*, 63 (1974); G. Konermann, Strahlentherapie *153*, 399 (1977); L.V. Slozhenikina, L.P. Mikhailets, L.A. Fialkovskaja and A.M. Kuzin, Bull. exp. Biol. Med. *86*, 939 (1978).

Increased release of tumour cells by collagenase at acid pH: A possible mechanism for metastasis

G.A. Turner[1]

University Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP (England),
1 March 1979

Summary. The ability of collagenase to disaggregate a solid metastasizing lymphosarcoma has been shown to considerably increase with reducing environmental pH. It is suggested that this effect may be operating in vivo to release cells from a primary tumour.

The detachment of cells from primary tumours is an important, but poorly understood, step in the metastatic cascade[2]. As a result of a chance observation, it became apparent that the efficiency with which collagenase could disaggregate a solid metastasizing lymphosarcoma increased with the lowering of the pH of the enzyme solution used. In view of the fact that intra-tumour pH is frequently found to be on the acid side[3], and that some evidence already exists to implicate collagenase in the release of primary cells into the circulation[4], it was thought that this effect could have significant bearing on this part of the metastatic process. This communication reports these findings.

Materials and methods. Phosphate buffer saline (PBS) solutions at pH 4.5, 5.3, 6.3 and 7.4 were prepared. Primary lymphosarcomas were removed from Syrian hamsters 18/20 days after implantation, and the excised tissue was roughly chopped, taking care to discard any necrotic on fibrous material. 4 aliquots of approximately 1 g were set up in glass universal bottles, and each aliquot was washed twice with 10 ml of its appropriate PBS by allowing the pieces of tumour to settle out and discarding the supernatant. Cell suspensions were prepared by stirring the chopped tissue for 1 h at 37 °C in 5 ml PBS (pH 4.5, 5.3, 6.3, or 7.4) containing 0.2 mg/ml collagenase (type II, Sigma). A stirring action was achieved using a glass-coated metal bar (15×2 mm) in the universal, placed on a magnetic stirring-base (Gallenkamp; setting 3). After treatment, any undisaggregated pieces were allowed to settle out, the supernatant was carefully decanted off, and 15 ml PBS (pH = 7.4) was added to the remaining pieces. The whole was resuspended and allowed to settle out again. The supernatant of the latter procedure was pooled with the first supernatant and the single cells pelleted by centrifiguration. Preliminary experiments had shown that this type of treatment was sufficient to remove over 95% of the single cells. The cell pellet was resuspended in 10–20 ml PBS (pH = 7.4) cell number was determined using an improved Neubauer counting chamber and cell viability was determined using a Trypan Blue method previously described[6].

Results and discussion. Lowering the pH of the collagenase solution considerable increased the yield of single cells for all the tumour preparations investigated (table 1). Statistical analysis of the data on a paired basis (Wilcoxon) indicated that the yield using PBS, pH = 6.3, was significantly greater ($p < 0.025$) than that using PBS, pH = 7.4. Furthermore, the yield using PBS, pH = 5.3, was significantly greater ($p < 0.025$) than that using PBS, pH = 6.3. There was no significant difference in the yields at pH 4.5 and 5.3. The pH of the PBS only had a significant effect ($p < 0.025$) on cell viability (table 2), if suspensions prepared using PBS pH 4.5 and 7.4 were compared.

It is unlikely that our results can be explained by an increase in the activity of the particular collagenase preparation we used because it has been shown that this type of preparation has optimum activity between pH 7 and 9[7]. What seems more feasible is that this change is brought about by the effect of the lowered pH on the tumour. It is well known that at acidic pH collagen becomes more susceptible to digestion by nonspecific collagenolytic en-