Mutagenicity Studies of Saccharin in Mice

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Saccharin is widely used as an artificial sweetener. By using the Basc method it was repeatedly found that saccharin induced sex linked recessive lethals in spermatozoa of Drosophila melanogaster and delayed mutations (mosaics in the X chromosome). At the concentration of 5×10^{-3} M of saccharin 1.36% sex-linked recessive lethals and 0.37% of mosaics were induced (ŠRÁM and WEIDENHOFFEROVÁ 1969, ŠRÁM and ZUDOVÁ 1972). At the concentration of 10×10^{-3} M saccharin induced 0.25% of whole body and 0.12% of mosaic mutations in the dumpy locus (ŠRÁM unpublished).

Saccharin also induced chromosome aberrations in Vicia faba (SAX and SAX 1968) and significantly increased the frequency of chromosome breaks and gaps in Chinese hamster cell line (KRISTOFFERSSON 1971).

From the toxicological point of view saccharin is a compound that is relatively rapidly excreted in the urine of animals and man - even 90% of very high doses are excreted in the urine in unchanged form within 24 hrs after application.

TAYLOR et al. (1968) performed an extensive study on mice, rats and dogs with saccharin per os or saccharin mixed with cyclamates without revealing any pathological change. Only exceptionally does saccharin induce allergy and vomiting in man.

As screening tests on Drosophila melanogaster and Vicia faba were positive, further studies on mutagenicity were performed to evaluate the genetic risk of saccharin.

METHODS

Dominant lethal test. The mutagenicity was tested in ICR male mice aged 10 weeks. The ICR males were injected intraperitoneally with a single dose or repeated doses of saccharin. Five groups of ICR males, each consisting of 22 or 25 animals, were treated with saccharin dissolved in isotonic solution. The control groups were treated with isotonic solution only.

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Design of experiment.

Group	Dose of saccharin in mg/kg BW	Administration time intervals in hrs	Number of males in the group
A	1000	-	22
B	5 x 200	24	22
C	5 x 50	12	25
D	5 x 100	12	25
E	5 x 200	12	25
controls	isotonic sol.	-, 12	22,25

After the last dose of saccharin each male was mated with two ICR females per week for 8 consecutive weeks.

All females were autopsied between day 13 and 15 of pregnancy and scored for corpora lutea (CL), total number of implants (I), early and late fetal deaths (R) and live embryos (I-R). Total dominant lethality was calculated according to the formula:

$$\frac{CL - (I-R)}{CL}$$

Pre-implantation lethality, CL - I/CL, and post-implantation lethality, R/I, were also calculated. In addition, the pregnancy index, P I, was determined for each experimental group, i.e., the percentage of mated females pregnant at the time of autopsy.

Differences in the relative frequencies were tested using the t-test and F-tests for homogeneity were carried out. Results obtained during weeks 1 to 3 correspond to postmeiotic stages of spermatogenesis, while results obtained during weeks 4 to 8 can be related to the premeiotic stages (ŠRÁM et al., 1970).

Cytological analysis of chromosome rearrangements.

Ten male mice from Group E (5 x 200 mg/kg at 12 hour intervals) and ten control mice were killed 12 weeks after the last treatment and meiotic preparations were made according to the method of MEREDITH (1969).

The testes of each animal were scored separately as to the presence of multivalent configurations or changes in the total number of chromosomes. From each testis 100 spermatocytes at the diakinesis-first metaphase stage of meiosis were examined, i.e. altogether 2000 spermatocytes in each analyzed group. The spermatocytes were classified according to the type of translocation and the number of chromosomes (ŠRÁM and ZUDOVÁ 1973).

The saccharin (sodium saccharin, sodium o-benzosulfamide, soluble, m.v. 241.2) was purchased from BDH, England.

RESULTS

Dominant lethal test. Group A - 1000 mg/kg BW (Table 1). This dose was not toxic to any males in this experiment. The only change induced was an increase in pre-implantation lethality during week 3 (Stage of early spermatids). The frequency of fertilization did not differ from that in the control group throughout the entire experiment.

TABLE 1

Induction of dominant lethals by saccharin

Week	PI	CL	I	R	I-R	CL-(I-R)/CL	CL-I/CL	R/I
					Group A (1000 mg/kg BW)			
1	0.7273	458	422	22	400	0.1266	0.0786	0.0521
2	0.7727	464	420	19	401	0.1358	0.0948	0.0452
3	0.7500	470	415	29	386	0.1787++	0.1170++	0.0699
4	0.5455	346	312	12	300	0.1329+	0.0983	0.0358
5	0.7045	432	403	26	377	0.1273	0.0671	0.0645
6	0.7273	458	406	14	392	0.1441	0.1135	0.0345
7	0.7045	455	410	22	388	0.1473	0.0989	0.0537
8	0.7273	474	438	23	415	0.1245	0.0759	0.0525
					Group B (5 x 200 mg/kg BW at 24 hr intervals)			
1	0.7955	505	441	34	407	0.1941++	0.1267+	0.0771
2	0.7045	430	397	10	387	0.1000	0.0767	0.0252
3	0.7045	432	394	22	372	0.1389	0.0880	0.0558
4	0.7500	459	394	24	370	0.1939++	0.1416++	0.0609
5	0.7045	434	385	19	366	0.1567+	0.1129++	0.0494
6	0.6364	399	360	27	333	0.1654	0.0977	0.0750
7	0.7500	450	408	36	372	0.1733	0.0933	0.0882
8	0.6364	383	351	14	337	0.1201	0.0836	0.0399
					Control			
1	0.7500	471	433	18	415	0.1189	0.0807	0.0416
2	0.7955	502	464	23	441	0.1215	0.0757	0.0496
3	0.7273	454	431	18	413	0.0903	0.0517	0.0418
4	0.7045	435	408	16	402	0.0759	0.0621	0.0392
5	0.7273	443	423	20	403	0.0903	0.0451	0.0473
6	0.6818	428	396	22	374	0.1262	0.0748	0.0556
7	0.7273	439	403	17	386	0.1207	0.0820	0.0422
8	0.7500	458	414	20	394	0.1397	0.0961	0.0483

$^{+}P = 0.01$

$^{++}P = 0.001$

TABLE 2

Induction of dominant lethals by saccharin

Week	PI	CL	I	R	I-R	CL-(I-R)/CL	CL-I/CL	R/I
				Group C (5 x 50 mg/kg BW at 12 hr intervals)				
1	0.8000	537	493	25	468	0.1285	0.0819	0.0507
2	0.7000	491	436	28	408	0.1690	0.1120	0.0642
3	0.7800	507	471	26	445	0.1223	0.0710	0.0552
4	0.8000	570	510	30	480	0.1579	0.1053	0.0588
5	0.8000	558	487	29	458	0.1792	0.1272	0.0595
6	0.7800	528	485	25	460	0.1288$_{+}$	0.0814$_{+}$	0.0515
7	0.8000	536	468	22	446	0.1679^{+}	0.1269^{+}	0.0470
8	0.7800	516	463	25	438	0.1512	0.1027	0.0540
				Group D (5 x 100 mg/kg BW at 12 hr intervals)				
1	0.8600	573	532	41	491	0.1431	0.0716	0.0771
2	0.7600	521	463	26	437	0.1612$_{++}$	0.1113$_{++}$	0.0562
3	0.8200	612	501	36	469	0.2337$_{++}$	0.1814$_{+}$	0.0639
4	0.8000	574	491	40	451	0.2143^{++}	0.1446^{+}	0.0815
5	0.7800	554	504	32	472	0.1480	0.0903	0.0635
6	0.8000	546	489	25	464	0.1502	0.1044	0.0511
7	0.7400	511	464	37	427	0.1644	0.0920	0.0797
8	0.7800	559	514	15	499	0.1073	0.0805	0.0292
				Group E (5 x 200 mg/kg BW at 12 hr intervals)				
1	0.9000	638	577	41	536	0.1599$_{++}$	0.0956$_{++}$	0.0711$_{++}$
2	0.8400	618	513	58	455	0.2638$_{++}$	0.1699^{+}	0.1131^{++}
3	0.7600	530	461	38	423	0.2019^{++}	0.1302^{++}	0.0824$_{++}$
4	0.8000	604	482	53	429	0.2897^{++}	0.2020^{++}	0.1100^{++}
5	0.7600	542	511	22	489	0.0978	0.0572	0.0431
6	0.8000	560	501	40	461	0.1768$_{++}$	0.1054$_{++}$	0.0798
7	0.7600	569	483	22	461	0.1898^{+}	0.1511^{++}	0.0455
8	0.7200	509	443	23	420	0.1749^{+}	0.1297^{++}	0.0519
				Control				
1	0.8200	564	522	24	498	0.1170	0.0745	0.0460
2	0.7000	536	491	22	469	0.1250	0.0840	0.0448
3	0.8200	568	520	25	493	0.1285	0.0845	0.0481
4	0.8000	549	498	23	475	0.1348	0.0929	0.0462
5	0.8000	582	527	25	502	0.1375	0.0945	0.0474
6	0.7800	552	502	21	481	0.1286	0.0906	0.0418
7	0.8000	543	502	23	479	0.1179	0.0755	0.0458
8	0.7800	512	477	22	455	0.1113	0.0684	0.0461

$^{+}P = 0.01$

$^{++}P = 0.001$

Group B - 5 x 200 mg/kg BW at 24 hr intervals (Table 1). Total dominant lethality and preimplantation lethality were increased in the premeiotic stage of spermatogenesis during weeks 1, 4 and 5. Male fertility was not affected.

Group C - 5 x 50 mg/kg BW at 12 hr intervals (Table 2). Results obtained during individual weeks did not differ from corresponding values in the control group. A statistically significant effect was observed only when pooled values for the premeiotic stage of spermatogenesis were examined.

Group D - 5 x 100 mg/kg BW at 12 hr intervals (Table 2). Total dominant lethality and preimplantation lethality were increased during the third and fourth weeks. Pooling the results of several weeks, total dominant lethality was increased throughout spermatogenesis. Pre-implantation lethality was increased during the postmeiotic stage of spermatogenesis only. Fertility did not change.

Group E - 5 x 200 mg/kg BW at 12 hr intervals (Table 2). The applied dose was not toxic during the experiment. This group showed the highest number of statistically significant differences from the control group. Total dominant lethality, preimplantation and postimplantation lethality were increased in the postmeiotic and premeiotic stages of spermatogenesis. Total dominant lethality and preimplantation lethality were increased in the 2nd, 3rd, 4th, 7th and 8th week, postimplantation lethality in the 2nd and 4th week. The fertility of treated males was not influenced.

Statistically significant F-values were found when the numbers of implantations and of live embryos per female were tested for homogenity among groups receiving a single dose or a series of fractionated doses. This difference also corresponded to the increase of preimplantation lethality in treated groups.

TABLE 3

The effect of 5 x 200 mg saccharin/kg BW on the induction of chromosome rearrangements in the spermatogonia of ICR mice

Classification of the spermatocytes		Treated group metaphases	%	Control group metaphases	%
20	II	1879	93.95	1987	99.35
18	II + R IV	4	0.2		
18	II + CH IV	26	1.3		
18	II + III + I	2	0.1		
19	II + X + Y	56	2.8	8	0.4
19	II + 2 I	14	0.7	3	0.15
19	II + I	19	0.95	2	0.1

Cytological analysis of chromosome rearrangements. Saccharin induced 6% chromosome abnormalities (Table 3): 1.6% translocations, 4.5% metaphases with separated X and Y chromosomes, 2 univalents or one univalent only. Comparing the results among separate males and their testes, the results were found to be heterogenous.

DISCUSSION

Saccharin induced higher ratios of dominant lethals in experiments with male mice. Mutation frequencies differed depending on whether a single dose or repeated doses were given and on what time intervals between doses were chosen. When saccharin was applied in the single dose of 1000 mg/kg BW, the increase was found as a stage of early spermatids only. When the same dose was divided into 5 doses at 12 hr intervals, the dominant lethality increased in the 2nd, 3rd, 4th, 7th and 8th week. When the interval between doses was increased to 24 hrs, increased dominant lethality was observed during weeks, 1, 4 and 5. The differences in responses between groups tested at 12 and 24 hr intervals may be explained by the higher heterogeneity of cells exposed in the latter case, because of the increase in total treatment time (between first and last dose) from 2 to 4 days.

Since the highest incidence of dominant lethality was observed after treatment at 12 hr intervals, instead of at 24 hr intervals, it was concluded that saccharin is quickly eliminated from the body, which may allow for recovery from changes induced following even very high doses.

Varying the interval between repeated doses, which may be specific for various types of cells, it was found that changes induced by additional doses may be confounded with premutational damage induced by previous doses. If the interval between doses is longer, the previous changes may partially disappear and therefore total damage may be smaller. A similar relationship was found also after fractionated application of TEPA (ŠRÁM and ZUDOVÁ 1973).

The relationship between the dose and dominant lethality was therefore studied after the repeated application at intervals of 12 hrs. Higher doses increased the frequency of dominant lethals. The dose of 5 x 50 mg/kg BW caused a minimal effect in the present experiment.

A similar increase of dominant lethals was also found when female mice were treated (ŠRÁM, unpublished).

Chromosome abnormalities induced at the stage of spermatogonia are an important result showing the ability of saccharin to induce transferable changes. These

changes correspond to the results of the dominant lethal test under identical treatment schedules.

The results of the dominant lethal test and cytological analysis of chromosome rearrangements proved the ability of saccharin to induce genetic damage at the stage of spermatogonia.

SUMMARY

Genetic effects of saccharin were studied in mice by the dominant lethal test and the cytological analysis of changes induced in the stage of spermatogonia formation.

Male mice were treated intraperitoneally with a single dose of 1000 mg/kg BW or repeated doses of 5 x x 200 mg/kg BW at 24 hr intervals, or 5 x 50 mg/kg BW, 5 x 100 mg/kg BW and 5 x 200 mg/kg BW at 12 hr intervals.

The highest frequency of dominant lethals was found in the group treated with 5 x 200 mg/kg BW at 12 hr intervals. Following the relationship between the dose and the frequency of dominant lethals, the incidence of dominant lethals increased with increasing dose levels of saccharin. A cytological analysis of chromosome rearrangements in spermatogonia revealed that a dose of 5 x 200 mg saccharin/kg BW given at 12 hr intervals produced 1.6% translocations and 4.5% separated X and Y chromosomes or univalents.

Summing up the results of the dominant lethal test and those of the cytological analysis of spermatocytes in mice with results obtained on Drosophila melanogaster, Vicia faba and Chinese hamster cell line, it is possible to conclude that saccharin is a mutagenic compound inducing both point mutations and chromosome aberrations.

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