SCEs INDUCTION IN GOATS: ANALYSIS OF METHODOLOGICAL VARIATION FACTORS

INDUCCIÓN DE INTERCAMBIO DE CROMÁTIDAS HERMANAS EN CAPRINOS: ANÁLISIS DE LOS FACTORES DE VARIACIÓN DE LA METODOLOGLA

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ADDITIONAL KEYWORDS

Sister chromatid exchanges. Lymphocytes. Goat.

PALABRAS CLAVE ADICIONALES

Cultivos linfocitarios. SCEs. Caprino.

SUMMARY

Several sources of variation in the SCE methodology were studied. Cell concentration, fetal sera, BrdU doses, and mytogens were analyzed to estimate the relative effect of each on the SCEs frequency.

As it was expected the BrdU concentration was the main source of variation. There was no evidence that the sera or mytogen had a significant effect. The initial cell inoculum muffling effect on the SCEs frequency could not be outed either.

The results pointed out that the BrdU effect could have obscured the action of the other factors, which indicates that only a very little of the total SCEs variation could be caused by these, and in any case it could be rejectable in standard analysis conditions.

RESUMEN

Se analizan diferentes fuentes de variación de la metodología de intercambio de cromátidas hermanas. La concentración celular, el tipo o clase de suero fetal, la dosis de 5-bromo-2 deoxiuridina (BrdU) así como los agentes mitógenos fueron evaluados para estimar su efecto sobre la frecuencia global de intercambios en cultivos linfocitarios caprinos.

Como se esperaba, la concentración de BrdU fue la principal fuente de variación. No se halló evidencia de que el suero y el mitógeno tuviesen un efecto significativo sobre esta frecuencia. Asimismo, no pudo comprobarse el efecto amortiguador de la concentración celular inicial.

Los resultados señalan que el efecto del BrdU podría oscurecer la acción de los restantes factores, lo cual indica que sólo una pequeña porción de la variación de la frecuencia de SCEs podría ser causada por estos y por lo tanto podría descartarse en condiciones estándar de análisis.

INTRODUCTION

The SCEs test is being widely used on domestic species. The studies are mainly focused on the SCEs frequency analysis as a suitable test to detect the carcinogenic

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and mutagenic effect of many drugs and agents.

Through this methodology the potential carcinogenic and mutagenic actions of some routinely used products in animal production like dimetil-benzatracene (DMBA); ciclosfosfamide; cloranfenicol; etil metanosulphonate; growth promoters as well as environmental mutagens has been evaluated (McFeee and Sherril, 1983; Catalan, 1991; Leibenguth and Thiel, 1986; Chrisman and Lasley, 1975; Rubes, 1987; Ianuzzi, 1988).

Although the SCEs test presents two desirable features low cost and simplicity, its unknown variation sources has been the main objection by several authors. In humans these variation sources have been reported to be exogenous factors (Wulff, 1990), endogenous, as the age of donants (Melaragno and Cardoso, 1990; Seshadri *et al.* 1982), or the intraindividual variations, (Lindblad and Lambert, 1981; Lambert *et al.*, 1982) which more recently has been related to the differences between T and B lymphocytes populations and the T lymphocytes subpopulations variation (Miller, 1991; Santesson, 1986; Santesson *et al.*, 1983).

Likewise a strong variation in the SCEs frequency produced by the methodology itself have been described in several works. The BrdU doses, cell culture media; fetal sera; mytogens; cell concentration; antibiotics; coagulants and the cell type could affect the basal frequency of in vitro SCEs, which indicates that it would be the most important source of error when the SCEs method is routinely used as a test. (Latt, 1974; Kato and Sandberg, 1974; Wollf and Perry, 1974; Morgan and Crossen, 1981; Lamberti *et al.*, 1981; Gosh and Nand, 1979; McFee and Sherril, 1979,1983; Menhert *et al.*, 1984; Tucker and Christensen, 1987; Tucker *et al.*, 1987; Bender *et al.*,1990a,b)

The SCEs frequency and distribution characteristics have been described in goats (López and Arruga, 1992) but in this as well as other domestic species there is few information about the methodological SCEs variation sources

The objective of the present work was to cover this information gap and to analyze the main factors that could affect the SCEs frequencies in lymphocyte cell cultures as an attempt to standarize the methodology.

MATERIALS AND METHODS

Twelve animals (6 males and 6 females) belonging to the pure breeds Blanca Celtibérica, Toggemburg and cross breeds were used.

A modified protocol from Moorhead *et al.* (1960) was used for lymphocyte cell cultures. Duplicate cell cultures were made from each animal in RPMI 1640 media (Gibco) supplemented with 1 p.cent Peniciline-Streptomicyne sol. (Gibco), and 1 p. cent of L-Glutamine (Sigma). The sera, mytogens, and cell concentration were changed according the experimental design performed.

An adaptation of the method of Perry and Wolff (1974) was used to detect sister chromatid exchanges. Briefly, whole blood cell cultures were made in RPMI media (as described above) and the BrdU (5-bromo-2-deoxyuridine, Sigma) doses were added 24 hs after. The cultures were harvested after two rounds of replication (48 hs) in presence of BrdU.

The harvest, cell fixation and chromosome spreads were made in standard conditions avoiding light exposure, and the FPG method (Fluorescent Plus Giemsa) was used to detect the differential sister chromatid staining.

The study was divided in three different blocks or sets to calculate block effect in a separately way from the rest of the factors. Four animals were used in each block, and four factors (BrdU doses, sera, mytogen and cell concentration) with four levels in each were evaluated as well:

- Cell concentration:
  - C1: $2x10^5$ lymphocytes/ml
  - C2: $3x10^5$ "
  - C3: $4x10^5$ "
  - C4: $5x10^5$ "

- BrdU doses:
  - D1: 1 µg/ml (Sigma)
  - D2: 2 " "
  - D3: 5 " "
  - D4: 10 " "

- Mytogens:
  - M1: Phytohaemaglutinin 10 UI (Wellcome, M form)
  - M2: Phytohaemaglutinin 5 UI + Pokeweed 5UI (Gibco)
  - M3: Phytohaemaglutinin 5 UI + Concanavaline A 5UI (Sigma)
  - M4: Phytohaemaglutinin 5 UI + Concanavaline A 5UI (Sigma)+ Pokeweed 5UI

- Fetal Sera:
  - S1: Fetal Calf Sera (Gibco) 10 p. cent
  - S2: 20 p. cent
  - S3: Artificial Fetal Sera (SeroMed) 20 p. cent
  - S4: Artificial Fetal Sera (SeroMed) 10 p. cent + Fetal Calf Sera 10 p. cent

An automatic cell counter was used to estimate the white blood cell fraction and the initial cell inoculum was adjusted in relation to the lymphocyte percentage detected by differential staining.

The SCEs frequency was estimated in thirteen (30) metaphase spreads/culture (60 cells/animal aprox).

The proliferation cell speed was estimated by the Proliferation Rate Index (Speit and During 1986; Tice *et al.* 1976), and the PRI mean value of two cultures was the reference data for each animal.

The data set was analyzed by ANOVA test (factorial, four way), and the Schefee test was employed in order to calculate the significance in each variable level

RESULTS

The mean SCEs/cell frequency did not show statistical significance between the three experimental blocks ($p>0.05$). The mean SCEs/cell frequency per block was 6.26; 6.52 and 7.58 (block 1,2 and 3

Table I. *SCEs/cell average related to BrdU concentration.* (Frecuencia de SCEs/cel en relación a la dosis de BrdU utilizada).

BrdU*	1μg/ml	2μg/ml	5μg/ml	10μg/ml
SCEs/cell	5.99	6.31	6.68	8.28

*μg/ml

Table II. *Relationship between PRI (Proliferation Rate Index) and BrdU concentration.* (Relación entre el Índice de Proliferación Celular (PRI) y la concentración de BrdU).

Dosis 1 (1 μg/ml)	Dosis 2 (2 μg/ml)	Dosis 3 (5 μg/ml)	Dosis 4 (10 μg/ml)
PRI: 2.24	PRI: 2.21	PRI: 2.1	PRI: 2.02

respectively) and a general mean value of 6.93 SCEs/cell was obtained.

This mean value does not differ statistically from the frequency in each block, neither from the SCEs frequencies previously detected in this species or in related species like cattle or sheep.

The Schefee test indicated that there were significant differences in at least one of the different tested levels within each factor.

BrdU dose

All BrdU doses were suitable to SCEs differentiation. Statistical differences ($p<0.05$) were estimated between concentrations as well:

D1 (1 μgr/ml) in relation to D4 (10 μg/ml), D3 (5 μg/ml) and D2 (2 μg/ml); D2 in relation to D4 and D3 in relation to D4.

The high BrdU doses are coincident with the high level of SCEs/cell in each block when the BrdU effect was considered alone. The mean comparison test showed the differences **(table I)**.

PRI (proliferation rate index)- BrdU interaction

Taking into account the BrdU doses effect on the cell proliferation the ANOVA test showed differences between D1 related to D4 and D2 to D4 ($p<0.05$). The cultures with D1 and D2 presented a high rate of cell proliferation, in comparison to D3 and D4 treated cultures, which indicates again a negative effect of BrdU concentration on cell proliferation rate **(table II)**. No relationships between SCEs frequency and PRI values was detected. The correlation and regression analysis indicate a negative tendency between these variables but without statistical significance ($p>0.05$).

Cell concentration

This variable showed statistical differences in two intermediate concentrations, C2 and C3, but the correlation and regression curves did not showed any relation with the PRI or the SCEs frequency.

Mytogen agent

The phytohaemaglutinin-concanavalin A combination was the only one in which differences were detected. The lowest SCEs frequency were observed when this mytogen combination was employed.

Considering the mitogen effect in relation to BrdU doses and SCEs frequency, there were no differences when all BrdU doses were evaluated together. The statistical differences between this combination and mytogen 1 (10 UI phytohaemaglutinin) and 2 (phyto-

haemaglutinin - pokeweed) arose only when the high BrdU dosis were extracted from the analysis.

No difference neither tendency between mitogens was observed when the PRI values and mytogens were related.

SERUM

Serum 3 (20 p.cent of artificial calf serum) showed statistical differences in relation to serum 2 (20 p.cent fetal calf serum) and serum 1 (10 p.cent fetal calf serum), but the ANOVA test did not show differences when the relation between serum, SCEs frequency and PRI values was analyzed. The correlation and regression analysis between serum and PRI values did not show significant results.

Besides the values extraction of the cultures in which 10ml of BrdU was added the calculated differences were not signifficative ($p>0.05$).

DISCUSSION

The fact that *in vitro* and *in vivo* SCEs basal frequencies are a combination of both, spontaneous and induced exchanges, has been documented before (Bianchi and Larramendy, 1979). According to these, in the works where BrdU has been used, the sister chromatid frequencies should be considered as a summatory of the exchanges produced by this drug and the spontaneous ones.

Some characteristics of the SCEs frequency variation in goat lymphocyte cell cultures are pointed out in this work. The BrdU doses have been detected like the main variation source, although this effect has been intensively studied it is possible to extract some conclusions

The 1mg/ml of BrdU was the lowest doses that allowed to detect the SCEs, which do not agree with the observed by McFee and Sherril (1983) in cattle and sheep. This dosis was capable enough to induce a good differential staining with the lowest SCEs values together.

The small differences (lees than 1 SCEs/cell) between 1, 2 or 5 mg/ml BrdU doses indicate that there is a BrdU range that permit to obtain together the differential staining and a low frequency.

The cell concentration muffling effect reported by Bender *et al.* (1992b), which should reduce the induced SCEs by BrdU, could not be checked out here. The cultures with both high cell concentrations and BrdU dosis showed a high SCEs frequency, which indicate that the BrdU effect was present regardless of the initial cell concentration inoculum.

The slow reduction of PRI (Cell Proliferation Rates Index) values when the BrdU doses increase is showing a negative tendency but without any important effect when BrdU doses ranging between 1 to 10 mg/ml.

This agree with the paper previously published by Giulotto et al (1980) and Lopez and Arruga (1992), about the independence between PRI and SCEs frequencies when low BrdU doses are added, and indicates that the relation between BrdU catabolism, its DNA incorporation and proliferation cell mentioned by Massad *et al.* (in Catalan, 1991) should be taken into account only when high BrdU doses are used.

The sera is another factor that has been indicated to have an specific effect on the proliferation cell rates and SCEs frequency together. As with the mytogen, there were no differences between the four different sera combinations and no

important effects on the SCEs and PRI values could be observed. For these two factors the possible explanation can be related to the fact that the differences between mytogens or between sera used are too small to be detected in the analysis performed, or the experimental statistical design is not able to detect these.

CONCLUSIONS

The BrdU was the most important source of variation, and its modulatory effect on the SCEs frequency without a significant action on cell proliferation rate is proved. Likewise, there are no differences when BrdU doses ranging from 1 to 5μg/ml are used, which permit to work with a more wide doses range in routine test.

The rest of the factors were very difficult to quantify, which disagree with the results previously published by Bender *et al.* (1992a,b); Miller (1991), McFee and Sherril (1983); Goto *et al.* (1978).

The differences between this and the above mentioned works about the SCEs methodological variation sources could lie in the fact that in these the different factors were analyzed in a separate way and mainly with constant high BrdU doses.

Here the four variation factors were analyzed jointly instead , which makes possible to detect the interactions as well as to evaluate the relative effect of each when are considered together. In our opinion it is a more reliable evaluation about what could happen in a culture when the routine SCEs test is used and what is the relative importance of each factor in the SCEs global frequency.

As we observed, the variation caused by the range of 1 to 5 μg/ml BrdU doses has been strong enough to obscure the other sources. This indicates that only a small percentage of the total variation could be caused by these, and in any case, it could be rejected in a normal experiment.

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Recibido: 7-6-96. Aceptado: 4-11-96.