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Relationship of Sister Chromatid Exchanges, Genotoxicity and Carcinogenicity: An SAR–based Analysis

Herbert S. Rosenkranz

Florida Atlantic University, Department of Biomedical Sciences, 777 Glades Road, Boca Raton, FL 33431–0991

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Relationship of Sister Chromatid Exchanges, Genotoxicity and Carcinogenicity: An SAR–based Analysis[#]

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Abstract

The ability of chemicals to induce sister chromatid exchanges (SCEs) in cultured cells has been taken as indicating a potential for causing cancers. Tests for the ability to induce SCEs are widely used and are part of guidelines for submissions to regulatory agencies. In the present study, based upon SAR analyses of 10,000 agents representative of the "universe of chemicals", it is shown that the induction of SCEs is not highly predictive of the ability to cause cancers. It is further shown that the reason for this lack of predictivity is due to the fact that SCEs can be induced through mechanisms unrelated to the carcinogenic process.

Keywords. SAR; structure–activity relationships; sister chromatid exchanges; genotoxicity; mutagenicity; carcinogenicity.

1 INTRODUCTION

The determination of the ability of chemicals to induce sister chromatid exchanges [SCE) *in vitro* has been included in batteries of short term assays designed to identify potential carcinogens. The rationale for the inclusion of SCE assays, based upon the paradigm that "carcinogens are mutagens" [1,2], was that it would allow the detection of agents that induced genotoxic events more complex than the point mutations detected in microbial assays and that it represented a higher phylogenetic order than the former [3–14]. However, as more short–term tests and long–term carcinogenicity assay results were released, it became evident that the induction of SCEs was not highly predictive of carcinogenicity and in fact did not increase the predictivity of the *Salmonella* mutagenicity alone [15–17]. Moreover, it was recognized that cancers in animals could be induced by non–genotoxic agents [18–20] and, thus, would be missed by mutagenicity/clastogenicity assays. Additionally, it was recognized that SCEs could be induced by non–genotoxic events [21,22] unrelated to cancer induction or progression and could, therefore, confound the predictivity of test

[#] The article is dedicated to Professor Milan Randić on the occasion of his 70th birthday. His leadership of our discipline deserves this recognition.

batteries that included it.

Still, the ability to induce SCEs is retained as one of the options in submissions to meet regulatory requirements regarding cancer hazards to gain approval for the manufacture, distribution or use of chemicals and therapeutic agents. This requirement may be based upon the empirical observation that the vast majority of recognized human carcinogens are genotoxicants [23–25] and the perceived association of the induction of SCEs with genotoxic events.

The present study was undertaken to investigate the role of the induction of SCEs in recognizing potential carcinogens using a recently developed and validated method designated the "chemical diversity approach" [26–29]. While earlier studies had shown that the induction of SCEs did not add to probabilistic predictions based solely upon the results of the *Salmonella* mutagenicity assay [15–17], that conclusion was based upon the results of the U.S. National Toxicology Program (NTP) cancer bioassays and related short term tests. The NTP database, with a 50% prevalence of carcinogens, is not necessarily representative of the "universe of chemicals." In fact, it has been suggested that less than 5–10% of all chemicals present a carcinogenic hazard [30,31]. The present analyses are based upon that broader population of chemicals.

2 MATERIALS AND METHODS

2.1 SAR Methodology

For these studies we used the CASE/MULTICASE SAR expert systems described previously [32–34]. Application of this methodology results in the development of 4 submodels, each of which is derived from a different algorithm useful for investigating different aspects of the biological phenomenon under consideration. The projections of the four individual submodels were integrated into a single prediction based upon Bayes' theorem [26,35]. In each instance the cut–offs used to predict the activity of the 10,000 chemicals (see below) were set to assure the positive (or negative) predictive power of the model was optimal.

Table 1. Predictivity of the SAR Models

Abbreviation	Phenomenon	Concordance[a]
CA	Carcinogenicity: Rodents	74%
Salm	Mutagenicity: *Salmonella*	85%
SOS	Error prone DNA repair (SOS Chromotest)	87%
Chr Ab	Induction of Chromosomal Aberrations	66%
SCE	Sister Chromatid Exchanges *in vitro*	71%
SCE Mo	Sister Chromatid Exchanges *in vivo*	83%
MLA	Mutagenicity: Mouse Lymphoma Cells	70%
Mnt	Induction of Micronuclei *in vivo*	81%
iGJIC	Inhibition of Gap Junctional Intercellular Communication	70%
UDS	Unscheduled DNA Synthesis	78%
3T3 Tx	Cell Toxicity: Balb/c–3T3	76%
HeLa Tx	Cell Toxicity: Hela	74%

[a] Concordance between experimental results and predictions of chemicals external to the SAR models

Each of the SAR models used herein had been characterized [34] with respect to its ability to predict the activity of chemicals external to the model (see Table 1).

2.2 SAR Models

The validated SAR models used for these studies have been described previously: inhibition of GJIC [36], mutagenicity in *Salmonella* [37–39], SOS DNA repair *(i.e*., Chromotest) [40,41], carcinogenicity in rodents (combination of results of bioassays conducted by U.S. National Toxicology Program [18] and of those analyzed by Gold and associates in the Carcinogenic Potency Data Base [42–46], cellular toxicity: cultured BALB/c–3T3 (clonal assay) [47] and HeLa: (dye assay) [48], induction of unscheduled DNA synthesis in rat hepatocytes [49], sister chromatid exchanges and chromosomal aberrations in cultured CHO cells [50], bone marrow micronuclei [51] *in vivo* sister chromatid exchanges [52] and of mutations at the thymidine kinase locus of cultured mouse lymphoma cells [53].

2.3 The Chemical Diversity Approach: Rationale

The procedure is based upon the premise that the mechanistic relationship between biological phenomena can be derived from knowledge of the prevalence of chemicals which give identical responses in assays designed to probe that relationship. Thus, at the time the electrophilic theory of cancer causation was recognized [54] and the dogma that "carcinogens are mutagens" [1] led to the development of surrogate tests for putative carcinogens, we discovered significant experimental overlaps using rodent carcinogens and genotoxicants. However, further studies clearly found that a significant number of non–mutagens also induced cancers in rodents. The basis of "non–genotoxic" carcinogenesis is still under active investigation but clearly it derives from a number of different mechanisms. Still, based upon the above premise, we should be able to gain a mechanistic insight into this phenomenon by evaluating the concordance, or lack thereof, between non–genotoxicants that induce cancers in rodents and agents that cause non–genotoxic phenomena (*e.g*. peroxisome proliferation, mitogenesis, binding to estrogen receptor). Thus, an evaluation of the toxicological profiles of a population of chemicals might reveal significant associations between "non–genotoxic" inducers of cancers and inducers of another toxicological phenomenon. The observed prevalence of chemicals that induce both phenomena could then be compared with the prevalence expected, if it is assumed that the two phenomena are unrelated (*i.e*. null hypothesis). If the observed prevalence is significantly greater than the expected one, then it can be concluded that the two phenomena are related to one another mechanistically. (Similarly, if the observed prevalence is significantly lower than the expected one, it suggests that the two phenomena are antagonistic with one another, *e.g*. they could compete for an active site).

In implementing such an approach, it became quickly obvious that there is a scarcity of experimental data on the same chemicals across a variety of endpoints. Hence, the significance of

the observed joint prevalences cannot be ascertained. The current approach was devised to overcome this shortcoming. It is based upon the availability of characterized and validated models describing structure–activity relationships (SAR). Moreover, while reliable databases of toxicological models, when available, are usually limited to 200–300 chemicals, the approach used herein predicts the toxicological profiles of 10,000 chemicals representative of the "universe of chemicals" [55]. While no SAR model is perfectly predictive, when applied to a population of 10,000 chemicals, provided the sensitivity and specificity are approximately equal, we can expect that the overall prevalence will reflect the true distribution. This in turn will allow a determination of the significance of the observed joint prevalences. The results of these analyses can be expressed as "Δ", the difference between the observed and the expected prevalences or as "100Δ/Expected", which is 100×Δ/Expected prevalence (Table 2).

Table 2. Relationships Between Error Prone DNA Repair and Mechanisms of Carcinogenicity

Analyses	Phenomena[a]	Observed	Expected	Δ	100Δ/Expected	P–value
1	SOS^+ & UDS^+	690	270	420	155.6	0.0003
2	SOS^+ & $iGJIC^+$	361	338	23	6.8	0.2
3	SOS^+ & CA^+	936	413	523	127	<0.0001
4	$iGJIC^+$ & CA^+	1151	915	236	25.8	<0.0001

[a] For abbreviations see Table 1

The approach can be used to confirm specific hypotheses (*e.g.* the electrophilic theory of cancer causation) as well as to generate new (knowledge–based) hypotheses driven solely by the data and the availability of appropriate SAR models. Thus the induction of error–prone DNA repair (SOS chromotest) as a consequence of exposure to a chemical agent is taken to indicate that the agent is a genotoxicant [40,41]. Because the vast majority of recognized human carcinogens are mutagens/genotoxicants [23–25,56], a positive response in that assay is taken to indicate that the inducer is associated with a certain carcinogenic risk. Analysis using the "chemical diversity approach" [26] indicates that the SOS chromotest is significantly associated with the induction of unscheduled DNA synthesis (UDS) in rat hepatocytes (Table 2, Analysis No. 1). This is evidenced by the significantly greater observed than expected (Δ = 420) joint prevalence of chemicals that induce both phenomena. This is not unexpected as the induction of UDS, like that of the SOS chromotest, is also a genotoxic event. On the other hand, there is no significant interaction between the SOS chromotest and the inhibition of gap junctional intercellular communication (iGJIC) (Table 2, Analysis 2). This presumably reflects the non–genotoxic nature of iGJIC [57–59].

There is also extensive overlap between the ability to induce the SOS chromotest and the ability of such (genotoxic) chemicals to induce cancers in rodents (Table 2, Analysis 3). This, in fact, reflects the genotoxic induction of cancers and provides justification for using the SOS chromotest to identify potential carcinogens. Mechanistically, this presumably reflects the electrophilic theory of cancer causation [54] as reflected in the mutagenic activation of oncogenes or the inactivation of

suppressor genes [60–62].

Finally, when the prevalences of chemicals that inhibit GJIC and induce cancers are examined (Table 2, Analysis 4) there was a significant increase in the observed when compared to the expected prevalence. This confirms the non–genotoxic basis of carcinogenesis. Taken together, Analyses 3 and 4 support the notion that there are two mechanisms of carcinogenesis or that these may be two sequential events (*i.e*. genotoxic initiation and non–genotoxic promotion/progression).

3 RESULTS AND DISCUSSION

An examination of the joint prevalences of chemicals which are potential inducers of SCEs, as well as of other toxicological phenomena, indicates (Table 3, Column 1) that the induction of SCEs is associated with a number of other mutagenic, genotoxic and clastogenic phenomena (Rows 2–8, 10), but not with the inhibition of gap junctional intercellular communication (Row 9), an epigenetic phenomenon *par excellence* [57]. The overall profile of SCE is quite similar, qualitatively, to that of the induction of mutations in *Salmonella* (Table 3, Column 2). It is noteworthy that both show significant joint prevalences for the induction of cellular toxicity in cultured mammalian cells (Rows 11,12), reflecting either non–specific toxicity of SCE–inducing agents and/or attacks of non–DNA nucleophilic targets by mutagenic/genotoxic electrophiles [63].

Table 3. Relationships Among Genotoxic and Cytogenetic Phenomena.[a] For Abbreviations See Table 1

Row		SCE^+ (1)	$Salm^+$ (2)	$iGJIC^+$ (3)	SCE^+ $Salm^+$ (4)	SCE^+ $Salm^-$ (5)	SCE^+ SOS^+ (6)	SCE^+ SOS^- (7)	SCE^+ UDS^+ (8)	SCE^+ UDS^- (9)	SCE^+ $iGJIC^+$ (10)
1	CA	**30**	**53**	**26**	**129**	**–31**	**201**	4	**106**	8	**69**
2	Salm	**37**		3			**265**	–2	**149**	6	**54**
3	SOS	**33**	**140**	7	**265**	**–81**			**163**	–4	–4
4	Chr Ab	**75**	**30**	**8**	**159**	**22**	**158**	**66**	**133**	**59**	**105**
5	SCE		**37**	3							
6	SCE Mo	**49**	**27**	–4	**96**	**19**	**103**	**43**	**75**	**41**	**49**
7	MLA	**45**	**51**	2	**152**	**–18**	**174**	**25**	**93**	**31**	**62**
8	Mnt	**39**	**24**	0	**85**	**11**	**91**	**33**	**64**	**32**	**39**
9	iGJIC	3	3		**54**	**–33**	–4	6	**35**	–6	
10	UDS	**22**	**82**	1	**149**	**–47**	**163**	0			**35**
11	3T3 Tx	**60**	**28**	**13**	**170**	–1	**169**	**44**	**135**	**39**	**70**
12	HeLa Tx	**29**	**49**	**17**	**166**	**–53**	**204**	–6	**112**	6	**53**

[a] The number in bold indicates that the difference between observed and expected prevalences have a significance of $p \leq 0.05$.

In order to evaluate these associations between the induction of SCEs and other toxicological phenomena, we used the co–association of SCE induction with the ability, or lack thereof, to induce mutations in *Salmonella* (Table 3, Columns 4 and 5). This is based upon the assumption that the induction of mutations in *Salmonella* is a paradigm for genotoxicity. In fact, "genotoxic" carcinogens often are defined operationally as mutagenic carcinogens [18,64]. Accordingly, we determined the prevalence of the combination of SCE inducers, *Salmonella* mutagens and a series

of toxicological endpoints and compared these to the same combinations wherein it was assumed that these were *Salmonella* non–mutagens. Such analyses would allow an allocation of the contributions of genotoxic and non–genotoxic mechanisms in the induction of SCEs.

The results clearly indicate that when the combination included *Salmonella* mutagens (Column 4), that there was a highly significant association with carcinogenicity (Row 1), as well as with other mutagenic, genotoxic and clastogenic endpoints, as well as with toxicity. On the other hand, when the *Salmonella* mutagenicity response was negative (Column 5), the association with carcinogenicity was abolished, as was the association with primarily genotoxic phenomena [induction of error–prone DNA repair, mutations at the thymidine kinase locus of cultured mouse lymphoma cells, induction of unscheduled DNA synthesis (UDS) in rat hepatocytes (Rows 3,7,10)]. On the other hand, although the association with clastogenic effects [chromosomal aberrations, the induction of micronuclei and SCEs *in vivo* (Rows 4,6,8)] was decreased, it was still significant even for *Salmonella* non–mutagens (Table 3). Clearly, the non–genotoxic induction of these phenomena is unrelated to carcinogenicity, since the latter joint prevalence became negative (Row 1). Qualitatively, similar results were obtained when the induction of SCEs was paired with the induction of error–prone DNA repair (SOS chromotest) (Columns 6 and 7) or the induction of UDS in rat hepatocytes (Columns 8 and 9) which are also considered to be primarily genotoxic events. This then establishes further the contribution of non–DNA damaging events to the induction of SCEs.

The present results confirm and extend the previous findings, based upon a more restricted population of molecules, that the induction of SCEs does not improve carcinogenicity prediction beyond the conclusions based solely upon the results of *Salmonella* mutagenicity tests [15–17]. Our analyses clearly show that when a chemical is non–mutagenic in *Salmonella*, a potential to induce SCEs is not related to a carcinogenic potential. These findings extend to the relationship between carcinogenicity and other primarily genotoxic activities (SOS chromotest, UDS). They indicate further that the conclusions reached with respect to the induction of SCEs apply to other clastogenic effects (the induction of chromosomal aberrations, SCEs *in vivo* and micronuclei), all of which appear to have non–genotoxic components [65].

In fact, some of the present findings of greatly decreased projected prevalence of "non–genotoxic" clastogenicities (*i.e.* significant antagonisms) suggest that some of these phenomena may actually interfere with the carcinogenic process by competing for common precursors or intermediates. In the present context, a potential for inducing SCEs coupled to a genotoxic potential (e.g. *Salmonella* mutagenicity, UDS induction, error–prone DNA repair) increases the reliability of carcinogenicity prediction but not the probability itself. Thus, a positive response in the SCE assay coupled to a positive genotoxic response (*i.e.* a positive *Salmonella*, UDS or SOS chromotest) reinforces the result of the genotoxicity assay and hence serves as a confirmatory result.

The present analysis is based upon the behavior of a population of molecules and is used to derive mechanistic conclusions, as well as to place the role of the SCE test for carcinogen prediction in context. As such, they can guide the health policy analysts who must devise strategies to protect the public from the hazards associated with xenobiotics [66]. However, our conclusions must not be taken as an indication that generating experimental data for individual chemicals is no longer necessary.

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