

Supplementary Materials

Article

Refinement of animal model of colorectal carcinogenesis through the definition of novel humane endpoints

Rita Silva-Reis¹, Ana I. Faustino-Rocha^{1,2}, Mariana Gonçalves¹, Catarina Castro Ribeiro¹, Tiago Ferreira¹, CM Ribeiro-Silva¹, Lio Gonçalves^{3,4}, Luís Antunes^{1,5}, Carlos Venâncio^{1,6}, Rita Ferreira⁷, Adelina Gama^{5,8}, Paula A. Oliveira^{1,7}

¹ Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

² Department of Zootecnics, School of Sciences and Technology, University of Évora, Évora, Portugal

³ Engineering Department, UTAD, Vila Real, Portugal

⁴ Institute for Systems and Computer Engineering, Technology and Science, Porto, Portugal

⁵ Department of Veterinary Sciences, UTAD, Vila Real, Portugal

⁶ Department of Animal Sciences, UTAD, Vila Real, Portugal

⁷ Associated Laboratory for Green Chemistry (REQUIMTE), Department of Chemistry, University of Aveiro (UA), Aveiro, Portugal

⁸ Animal and Veterinary Research Center (CECAV), UTAD, Vila Real, Portugal

* Correspondence: pamo@utad.pt

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Table S1. Initial and final corrected BW, food and water intake in the first and last weeks of the experimental protocol. All data is presented as mean \pm S.D.

Group	Body weight (g)		Food consumption (g)		Water consumption (ml)	
	Initial	Final	Initial	Final	Initial	Final
CTRL1 (n=6)	223.4 \pm 10.2	409.0 \pm 19.7	20.7 \pm 0.2	26.3 \pm 0.2	29.7 \pm 2.6	26.3 \pm 0.7
CRC1 (n=8)	222.4 \pm 4.3	366.4 \pm 38.9 ^a	23.2 \pm 0.7	25.6 \pm 1.7	25.9 \pm 4.5	20.9 \pm 3.8
CTRL2 (n=6)	229.1 \pm 14.6	441.1 \pm 34.8	23.7 \pm 0.1	27.3 \pm 1.1	29.6 \pm 0.5	23.0 \pm 0.7
CRC2 (n=9)	225.8 \pm 7.9	393.9 \pm 36.7	23.8 \pm 0.4	27.8 \pm 0.6	28.1 \pm 0.9	20.9 \pm 1.9

^a Statistically different from group CTRL2 ($p < 0.05$). Control groups (CTRL1 and 2) injected with EDTA-saline and induced groups (CRC1 and 2). Groups 1 and 2 were euthanized 11 and 17 weeks after the first administration, respectively.

Figure S1. Animals' mean body weight *per week*. Control groups (CTRL1 and 2) injected with ethylenediamine tetraacetic acid (EDTA)-saline and induced groups (CRC1 and 2) injected with 1,2-dimethylhydrazine (DMH). Groups 1 and 2 were euthanized 11 and 17 weeks after the first administration, respectively.

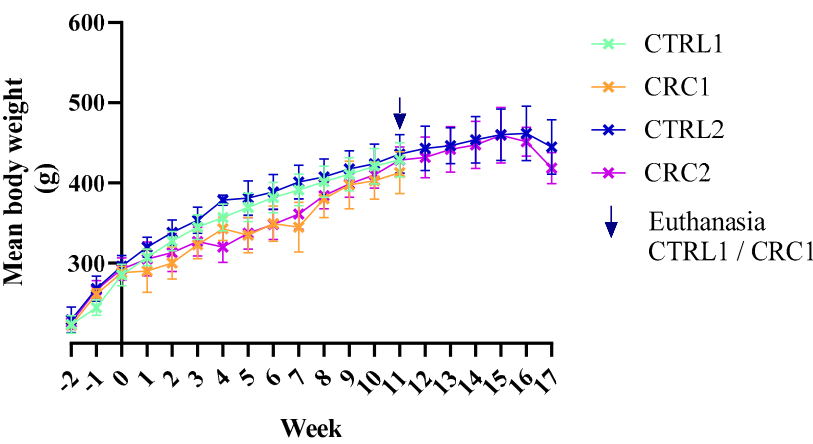


Figure S2. Animals' mean back and abdominal body temperature *per week*. Control groups (CTRL1 and 2) injected with ethylenediamine tetraacetic acid (EDTA)-saline and induced groups (CRC1 and 2) injected with 1,2-dimethylhydrazine (DMH). Groups 1 and 2 were euthanized 11 and 17 weeks after the first administration, respectively.

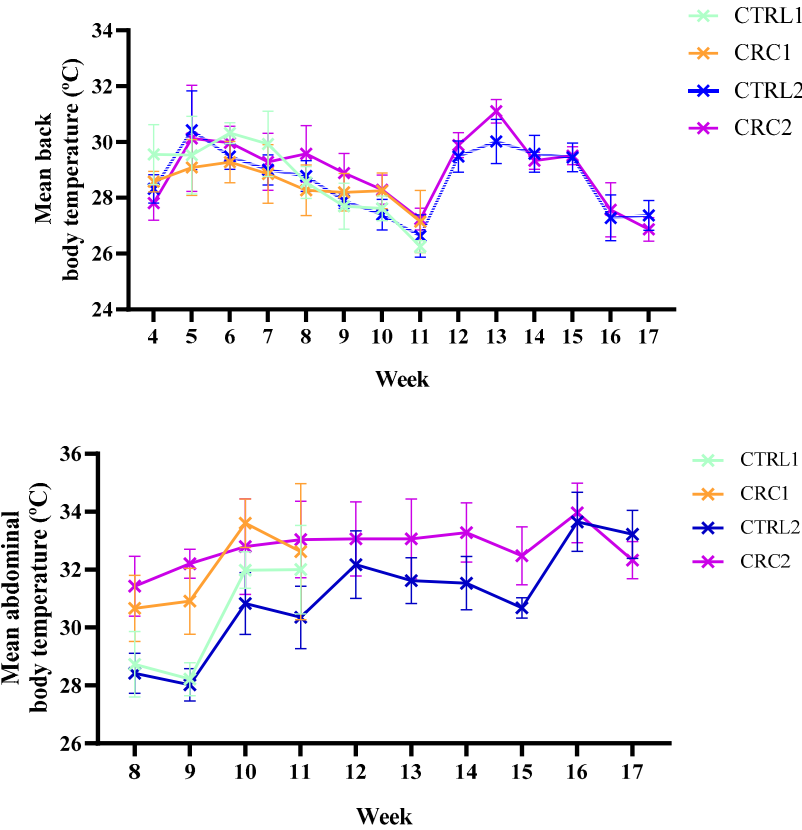


Table S1. Individual score assigned to each animal *per week*.

Group	Rat	Individual HE score per week																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17					
CTRL1	1	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d											
	2	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d											
	3	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d											
	4	1 ^d	1 ^d	0	0	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d						1 ^d					
	5	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d						1 ^d					
	6	0	0	0	0	0	0	0	0	0	1 ^d	1 ^d											
CRC1	1	0	0	0	0	0	1 ^g	2 ^{b, g}															
	2	0	0	0	0	0	0	0	1 ^d	1 ^d	1 ^d	1 ^d											
	3	0	0	0	0	0	2 ^{a, j}	2 ^{a, j}	2 ^{a, j}	3 ^{a, d, j}													
	4	3 ^{b, d, c}	0	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d											
	5	0	0	0	0	0	0	1 ^d	1 ^d	1 ^g	2 ^{a, g}	1 ^a											
	6	0	1 ^d	0	1 ^d	1 ^d	1 ^d	0	0	0	0	0											
	7	0	0	0	0	0	0	0	0	0	1 ^d	0											
	8	0	1 ^d	1 ^d	1 ^d	0	0	1 ^d	1 ^d	1 ^d	2 ^{a, d}	2 ^{a, d}											
CTRL2	1	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d					
	2	0	0	0	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d					
	3	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d					
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
	5	0	0	0	1 ^d	0	0	0	0	0	0	0	0	0	1 ^d	1 ^d	1 ^d	1 ^d					
	6	0	0	0	0	0	0	0	0	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d					
CRC2	1	0	0	0	0	0	0	0	0	1 ^d	2 ^{a, d}	3 ^{a, d, j}	3 ^{a, d, j}	3 ^{a, d, j}	3 ^{a, d, j}								
	2	1 ^d	0	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	2 ^{d, g}	1 ^d	1 ^d	1 ^d	2 ^{a, d}	3 ^{a, d, j}						
	3	1 ^d	0	3 ^l	3 ^l	0	0	2 ^{d, j}	2 ^{d, j}	2 ^{d, j}	2 ^{d, g}	2 ^{d, g}	0	0	0	1 ^d	1 ^d	1 ^d					
	4	0	0	0	0	0	0	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	2 ^{d, g}	2 ^{d, g}	2 ^{d, g}					
	5	0	1 ^d	1 ^d	0	0	0	0	0	0	0	2 ^{d, g}	2 ^{d, g}	2 ^{d, g}	1 ^d	1 ^d	1 ^d	1 ^d					
	6	0	0	0	1 ^d	1 ^d	2 ^{d, g}	0	0	1 ^d	1 ^d	2 ^{d, g}	1 ^d	0	1 ^d	1 ^d	1 ^d	1 ^d					
	7	0	0	0	1 ^d	1 ^d	2 ^{d, g}	0	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	1 ^d	2 ^{a, d, j}					
	8	0	1 ^d	1 ^d	1 ^d	1 ^d	2 ^{a, d}	2 ^{a, d}	1 ^a	2 ^{a, d}	2 ^{a, d}	2 ^{a, d}	2 ^{a, d}	2 ^{a, d}	2 ^{a, d}	3 ^{a, d, j}	3 ^{a, d, j}	3 ^{a, d, j}					
	9	1 ^d	1 ^g	1 ^d	1 ^d	2 ^{d, g}	1 ^d	1 ^d	2 ^{a, d}	2 ^{a, d}	3 ^{a, d, j}	3 ^{a, d, j}	3 ^{a, d, j}	3 ^{a, d, j}									

^a Body condition; ^b Body weight; ^c Posture; ^d Hair appearance and grooming; ^e Mucous Color; ^f Eyes, ears and whiskers; ^g Mental Status; ^h Response to external stimuli; ⁱ Hydration status; ^j Stool appearance; ^k Convulsions; ^l Dimension of damage caused by injections; ^m Macroscopic appearance of induced skin lesions; ⁿ Infection of induced skin lesions. Control groups (CTRL1 and 2) injected with ethylenediamine tetraacetic acid (EDTA)-saline and induced groups (CRC1 and 2) injected with 1,2-dimethylhydrazine (DMH). Groups 1 and 2 were euthanized 11 and 17 weeks after the first administration, respectively.

Table S3. New Scoring sheet for colorectal carcinogenesis chemically-induced by 1,2-dimethylhydrazine (DMH). Adapted from CCAC guidelines, Mason et al. (2004), Oliveira et al. (2017), Faustino-Rocha et al. (2019) [1–3]. Parameters added after this work are demarked in bold.

	Parameter	Score			
		0	1	2	3
General appearance and state of consciousness of the animal	Body condition	Good	Underconditioned	Emaciated	Swollen abdomen and priapism
	Body mass*	Normal	Loss/ Gain 5-10%	Loss/ Gain 10-20%	Loss/ Gain >20%
	Posture	Normal posture	Posture changes (hunched posture)	---	---
	Aspect of hair and grooming	Normal	Lack of grooming	---	---
	Chromodachryorrhea	Absence	Slight	Noticeable	Well-marked
	Abdominal Temperature	To be determined in a future work.			
	Mucous color	Normal	Slightly anemic	Moderately anemic	Severe anemia
	Grimace changes	Normal	---	Partially closed eyes, droopy ears, forward whiskers	Completely closed eyes, droopy and curved ears, forward and bunched whiskers
	Mental status	Normal	Slight apathy	Inactive	Stupor / Coma
	Response to external stimuli	Normal	Moderate response	Moderate response with vocalization	Violent response
Clinical signs	Hydration status	Normal	---	Abnormal skin pinch test (> 2 sec.)	---
	Appearance of stool	Normal	Diarrhea	Black (digested blood)	Bloody Stool

Convulsions	Absence	---	---	Presence
Dimension of damage caused by injections	Absence	Lesion with diameter ≤ 8 mm	Lesion with diameter ≥ 9 and ≤ 14 mm	Lesion with diameter ≥ 15 mm
Macroscopic appearance of induced skin lesions	Absence	---	---	Presence of necrosis
Infection of induced skin lesions	Absence	---	---	Presence of inflammatory exudate

* The body weight parameter must consider the body weight increase due to growth, being adjusted for each study

Recommendation - A total clinical score of 6 or a score of 3 in a single parameter indicates the need of urgent euthanasia.

References

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3. Oliveira, M.; Nascimento-gonçalves, E.; Silva, J.; Oliveira, P.A.; Ferreira, R.; Antunes, L.; Arantes-rodrigues, R.; Faustino-rocha, A.N.A.I. Implementation of Human Endpoints in a Urinary Bladder Carcinogenesis Study in Rats. *In Vivo (Brooklyn)*. **2017**, *31*, 1073–1080, doi:10.21873/invivo.11172.