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CCR

CCR · Cytotest Cell Research GmbH & Co. KG

CCR PROJECT 218913

IN VIVO / IN VITRO

UNSCHEDULED DNA SYNTHESIS IN

RAT HEPATOCYTES

WITH

LGH 10183/1

REPORT

CCR · In den Leppsteinswiesen 19 · D-6101 Roßdorf · F.R.G.
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CONTENTS

PREFACE

GENERAL

Sponsor:

Study Monitor:	Dr. M. Bracher
Testing Facility:	C C R CYTOTEST CELL RESEARCH GMBH & CO. KG D-6101 Roßdorf, F.R.G.
Evaluation of microscopic slides:	LMP LABORATORY FOR MUTAGENICITY TESTING Technical University of Darmstadt D-6100 Darmstadt, F.R.G.
CCR Project No.:	218913
Test Article:	LGH 10183/1
Title:	In vivo / in vitro Unscheduled DNA Synthesis in Rat Hepatocytes with LGH 10183/1

PROJECT STAFF

Director of CCR:	Dr. H.-E. Knoell
Scientific Consultant:	Prof. Dr. Herbert G. Miltenburger
Study Director:	Dipl. Biol. Rolf Fautz
Management:	Dr. Wolfgang Völkner

SCHEDULE

Start of Experiment	: January 28, 1991
End of Experiment	: June 19, 1991
Date of Protocol	: January 23, 1991
Date of Draft	: July 08, 1991
Date of Report	: August 14, 1991

PROJECT STAFF SIGNATURES

Study Director — Dipl. Biol. Rolf Fautz

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Date: August 14, 1991

Management: — Dr. Wolfgang Völkner

.....

Date: August 14, 1991

QUALITY ASSURANCE

The study was performed in compliance with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated March 14, 1990 (BGBL. I S. 521).

"OECD Principles of Good Laboratory Practice", Paris, 1981

GUIDELINES

There are no OECD and EEC guidelines and recommendations available to this test system.

ARCHIVING

C C R, D-6101 Roßdorf/F.R.G. will archive the following data for 30 years: Raw data, protocol and copy of report.

The following specimen and samples will be archived for at least 12 years:
Sample of the test article, slides.

No raw data or material relating to the study will be discarded without the sponsor's prior consent.

STATEMENT OF COMPLIANCE

Project Number: 218913

Test Article : LGH 10183/1

Study Director: Dipl. Biol. Rolf Fautz

Title : In vivo / in vitro Unscheduled DNA Synthesis in Rat Hepatocytes with LGH 10183/1

To the best of my knowledge and belief, the study was conducted in compliance with Good Laboratory Practice Regulations. This statement does not include the stability and the expiration date of the test article.

Study Director

C C R
Dipl. Biol. Rolf Fautz

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Date: August 14, 1991

QUALITY ASSURANCE UNIT

C C R, Cytotest Cell Research GmbH & Co. KG,
In den Leppsteinswiesen 19, D-6101 Roßdorf, F.R.G.

STATEMENT

Project Number: 218913

Test Article : LGH 10183/1

Study Director: Dipl. Biol. Rolf Fautz

Title : In vivo / in vitro Unscheduled DNA Synthesis in Rat Hepatocytes with LGH 10183/1

This report was audited by the Quality Assurance Unit and the study procedures were inspected on the following dates.

Dates of QAU Inspections/ Audits	Dates of Reports to the Study Director and to Management
January 25, 1991	January 25, 1991
February 13/14, 1991	February 14, 1991
March 12/15, 1991	March 15, 1991
April 11, 1991	April 11, 1991
May 14, 1991	May 14, 1991
July 17, 1991	July 17, 1991

Head of Quality Assurance Unit: Dipl. Biol. Ch. Bonk-Kassner

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Date: August 14, 1991

SUMMARY

The test article LGH 10183/1 was assessed in the in vivo/ in vitro UDS assay for its potential to induce DNA repair (UDS) in the hepatocytes of rats.

The test article was formulated in aqua bidest.. This suspending agent was used as negative control. The volume administered orally was 10 ml/kg body weight (b.w.). After a treatment period of 4 and 16 hours, respectively, the animals were narcotized and sacrificed by liver perfusion. Primary hepatocyte cultures were established and exposed for 4 hours to ^{3}HTdR which is incorporated if UDS occurs (3).

The test article was tested at the following dose levels:

4 hour treatment period: 750 mg/kg b.w.
16 hour treatment period: 75 and 750 mg/kg b.w.

For each dose level, including the controls, hepatocytes from three treated animals were assessed for the occurrence of UDS.

No toxic reactions of the animals occured at any of the treatment periods or dose groups. In addition, neither the viability nor the in vitro attachment of the hepatocytes was dramatically affected due to the in vivo pre-treatment with the test article.

No dose level of the test article revealed UDS induction in the hepatocytes of the treated animals as compared to the current negative controls.

An appropriate reference mutagen (2-AAF, 100 mg/kg b.w.) was used as positive control. Treatment with 2-AAF revealed distinct increases in the number of nuclear and net grain counts.

CONCLUSION

In conclusion, it can be stated that during the study described and under the experimental conditions reported, the test article did not induce DNA-damage leading to repair synthesis in the hepatocytes of the treated rats.

Therefore, LGH 10183/1 is considered to be non-effective in this in vivo/in vitro UDS test system.

OBJECTIVE

AIMS OF THE STUDY

This in vivo experiment was performed to assess the potential of the test article to induce DNA repair synthesis (UDS) in the hepatocytes of rats.

REASONS FOR THE STUDY

The UDS test is an assay for the detection of chemically induced effects on DNA. Lesions in DNA produced by chemicals may lead to genetic changes (mutations) through mis-repair.

Rats were exposed to the test article by an appropriate route. After a treatment period of 4 hours or 16 hours, respectively, the animals were narcotized and sacrificed by means of liver perfusion. Primary hepatocyte cultures were established and exposed for 4 hours to ^{3}HTdR which is incorporated if UDS occurs (3).

The UDS test measures the DNA repair synthesis after excision and removal of a stretch of DNA containing the region of damage induced by chemical or physical agents. The test is based commonly on the incorporation of tritium labelled thymidine (^{3}HTdR) into the DNA of mammalian cells which are not in the S-phase of the cell cycle. The uptake of ^{3}HTdR in cells isolated from rats exposed in vivo is determined by autoradiography.

The autoradiography approach involves a short in vitro culturing period of hepatocytes from treated rats on cover slips and exposing them to medium containing ^{3}HTdR. At the end of that period the cultures are processed for autoradiography and the amount of incorporated radioactivity is determined by silver grain counting. The few cells undergoing replicative DNA synthesis can be recognized by their heavy labelling in the autoradiographs and they are excluded from counting.

To investigate the time course of the test article the highest dose level is tested at two different exposure times.

To validate the test a reference mutagen is tested parallel to the test article.

MATERIALS AND METHODS

THE TEST ARTICLE

Name:	LGH 10183/1
Batch No.:	Rob 006958
Aggregate state at RT:	solid
Colour:	grey to blue
Molecular weight:	298.2
Purity:	> 99.6 % HPLC; approx. 120 ppm 2.4-Diaminoanisol
Analysis:	NMR, MS
Stability:	Pure: not indicated by the sponsor In solvent: oxidizes in solvent especially at pH > 7
Storage:	room temperature, light protected
Expiration date:	not indicated by the sponsor

On the day of the experiment (immediately before treatment), the test article was formulated in aqua bidest.. The vehicle is chosen according to its relative nontoxicity for the animals. All animals receive a single standard dose volume orally.

THE CONTROLS

The Negative Control

Name:	aqua bidest.
Route and frequency of administration:	singly, orally
Volume administered:	10 ml/kg b.w.

The Positive Control Substance

Name:	2-AAF; 2-Acetylaminofluorene
Supplier:	FLUKA FEINCHEMIKALIEN GmbH D-7910 Neu-Ulm, F.R.G.
Catalogue no.:	00300
Dissolved in:	dimethylsulfoxide/polyethylene glycol (1 + 9)
Dosing:	100 mg/kg b.w.
Route and frequency of administration:	singly, orally
Volume administered:	10 ml/kg b.w.

Solution prepared on day of administration.

The stability of the positive control substance in vehicle was unknown, but a DNA repair response in the expected range means biological stability demonstration.

THE TEST SYSTEM

Reasons for the Choice of the Experimental Animal Species

The rat is an animal which has been used for many years as suitable experimental animal in genotoxicity investigations. There are many data available from such investigations which may be helpful in the interpretation of results from the UDS test. In addition, the rat is an experimental animal in many physiological, pharmacological, and toxicological studies. Data from such experiments may also be useful for the design and the performance of the UDS test.

Strain:	Wistar/WU
Source:	SAVO-Ivanovas, med. Versuchstierzuchten GmbH D-7964 Kisslegg, F.R.G.
Number of animals:	25 (males)
Acclimatization:	minimum 5 days
Initial body weight at start of treatment:	approximately 160 - 180 g

According to the suppliers assurance the animals were in healthy condition. The animals underwent quarantine in the animal house of CCR for at least one week after their arrival. During this period the animals must not show signs of illness or altered behaviour.

The animals were distributed into the test groups at random and identified by cage number.

Husbandry

The animals were kept conventionally. The experiment was conducted under standard laboratory conditions.

Housing:	single
Cage type:	Makrolon Type I, with wire mesh top (EHRET GmbH, D-7830 Emmendingen 14, F.R.G.)
Bedding:	granulated soft wood bedding (ALTROMIN, D-4937 Lage/Lippe, F.R.G.)
Feed:	pelleted standard diet (ALTROMIN 1324, D-4937 Lage/Lippe, F.R.G.)
Water:	tap water, ad libitum (Gemeindewerke, D-6101 Roßdorf, F.R.G.)
Environment:	temperature 21 ± 3°C relative humidity 30-70% artificial light 6.00 a.m. - 6.00 p.m.

EXPERIMENTAL PERFORMANCE

Pre-Experiment for Toxicity

A prelimary study on acute toxicity to find an appropriate dose range for the main experiment was performed.

Dose Selection

For genotoxicity investigations it is generally recommended to use the maximum tolerated dose or the highest dose that can be formulated and administered reproducibly. The volume to be administered should be compatible with physiological space available.

The maximum tolerated dose is determined to be the dose that causes toxic reactions without having major effects on survival within 24 hours. If no toxic reactions are observed the highest dose to be used should be 1000 mg/kg b.w. (3).

Study Procedure

Test groups:

Five male rats are assigned to each test group. The animals are identified by their cage numbers as shown below in the table.

Test group	hours post-treatment 4 h	hours post-treatment 16 h
Negative control		6 - 10
Low dose		11 - 15
High dose	1 - 5	16 - 20
Positive control		21 - 25

Treatment

The animals were starved overnight (4 hours treatment) or approximately 6 hours (16 hours treatment) before receiving the test article, water was available continuously. At the beginning of the treatment the animals were weighed and the individual volume to be administered was adjusted to the body weight of the animals. The animals received the test article once. Five animals (males) were treated per dose group.

Isolation of the Primary Hepatocytes

The animals were sacrificed by liver perfusion. After anesthetizing the rats with 1.5 ml/kg b.w. Hypnodil (Janssen, D-4040 Neuss, F.R.G.) i.p. the liver was perfused through the vena portae with Hank's balanced salt solution (HBSS, Gibco/BRL, D-7514 Eggenstein, F.R.G.) supplemented with collagenase (0.05 % w/v, Boehringer Mannheim, D-6800 Mannheim, F.R.G.) adjusted to pH 7.4 and maintained at 37° C.

The hepatocytes were isolated from the liver and washed twice with HBSS. The crude cell suspension was filtered through a 94 µm stainless steel mesh to yield a single cell suspension. The quality of the actual performed perfusion was determined by the trypan blue dye exclusion method. In addition the number of the isolated cells was determined.

Culture Conditions

The washed hepatocytes were centrifuged and transferred into Williams medium E (WME, Gibco/BRL, D-7514 Eggenstein, F.R.G.) supplemented (1) with:

Hepes	2.38 mg/ml	Glutamin	0.29 mg/ml
Penicillin	100 units/ml	Insulin	0.50 µg/ml
Streptomycin	0.10 mg/ml	Fetal Calf Serum	100 µl/ml

The medium without the cells was adjusted to pH 7.6.

At least five cultures were established for each animal. Aliquotes of 2.5 ml with freshly isolated hepatocytes in complete culture medium (1.0×10^5 cells/ml) were added to 35 mm six-well cluster dishes (Greiner, D-7440-Nürtingen, F.R.G.) containing one gelatinized 25 mm round plastic coverslip (Thermanox, Flow Laboratories, D-5309 Meckenheim, F.R.G.) per well.

After an attachment period of approximately 1.5 h in a 95 % air/ 5 % CO_2 humidified incubator at 37° C the culture medium was discarded. Then the cell layer was rinsed once with PBS to remove non-adherent cells (11). Subsequently ^{3}HTdR (5 µCi/ml, specific activity 20 Ci/mmol; New England Nuclear, D-6072 Dreieich, F.R.G.) in 2.0 ml culture medium (WME, 1% FCS) was added to the cultures. After a labelling time of 4 h the cells were washed twice with WME supplemented with 1 % FCS and 0.25 mM unlabelled thymidine. Cultures were incubated overnight using the same medium (3). To prepare for autoradiography the medium was replaced by a hypotonic solution of 1 % sodium citrate for 10 minutes to swell the nuclei for better grain quantification (11). The cells on the coverslips were then fixed by three changes of methanol:acetic acid (3+1 v/v) for 15 minutes each, rinsed with 96 % ethanol, and air dried.

Three of the cultures from each animal were used for the UDS assay. Two cultures were used for determination of cytotoxicity and attachment efficiency with the neutral red assay (2).

Autoradiographic Processing

The cover slips were mounted the side carrying the cells up on glass slides and coated with ILFORD K-2 (Ilford, D-6078 Neu-Isenburg, F.R.G.) photographic emulsion in the dark. The coated slides were stored in light-proof boxes in the presence of a drying agent for 12 -14 days at 4° C. The photographic emulsion is then developed with KODAK D-19 (Kodak, D-7000 Stuttgart, F.R.G.) at room temperature, fixed in TETENAL (Tetenal, D-2000 Norderstedt, F.R.G.) and stained with 0.4 % aceto orcein.

Quantification of UDS

Evaluation was performed microscopically on coded slides using NIKON microscopes with 100 x oil immersion objectives. The number of silver grains above the nucleus was counted automatically using the ARTEK 880 counter. In addition, the number of grain counts of one nuclear-sized cytoplasm adjacent to the nucleus was counted (3). At least two slides per animal and 50 cells per slide were evaluated. Heavily labelled S-phase cells were excluded from counting.

Three animals per group were evaluated as described above. The two remaining animals per test group would be evaluated if an animal dies spontaneously or in case of technical problems concerning the isolation of the hepatocytes.

DATA RECORDING

The data generated were recorded in the laboratory protocol. The results were presented in tabular form, including experimental groups with the test article, negative and positive controls.

The nuclear and cytoplasm grain counts were reported separately (5). The mean counts with standard deviation were used to describe the distribution of ^{3}HTdR incorporation in the nucleus and the cytoplasm.

EVALUATION OF RESULTS

A test article is classified as positive if it induces either a statistically significant dose-related increase in radiolabel incorporation expressed as grains per nucleus or a reproducible and statistically significant positive response for at least one of the test points.

A test article producing neither a statistically significant dose related increase in radiolabel incorporation expressed as grains per nucleus nor a statistically significant and reproducible positive response at any one of the test points is considered non-effective in this system.

Statistical significance can be evaluated by means of the non-parametric Mann-Whitney test (4).

However, both statistical and biological significance should be considered together.

BIOMETRY

A statistical evaluation of the results was not necessary to perform as the number of nuclear and net grain counts of the groups treated with the test article were in the range of the corresponding controls.

RESULTS

PRE-EXPERIMENT

A series of pre-experiments was performed to find an adequate dose. After treatment with 1000 mg/kg b.w. 2 out of 5 treated amimals died after 24 h. The surviving animals showed severe toxic symptoms: reduction of spontaneous activity, eyelid closure, and pilo erection.

After treatment with 750 mg/kg b.w. all animals survived for at least 24 h and hepatocytes isolated from these animals yielded viabilities sufficient for the UDS-assay. However, due to the chemical treatment the kidneys, the urine, and the liver of the animals were dark coloured. In addition, the liver was relative solid.

After treatment with 500 mg/kg b.w. the kidneys and the urine were coloured, but the liver was unaffected.

According to the results of the pre-experiments 750 mg/kg b.w. was chosen as the highest dose for the UDS-assay.

TABLES OF RESULTS

VIABILITY AND ATTACHMENT OF THE HEPATOCYTES

Test Article: LGH 10183/1

Dose [mg/kg b.w.]	Treatment period	Animal No.	Viability[1] [%]	Number of isolated cells [x 10^6]	Attachment [O.D. 540 nm][2]
750	4h	2	72	344	0.710
	4h	3	71	282	0.495
	4h	4	69	336	0.951
Vehicle	16h	6	81	179	0.619
	16h	7	83	200	0.510
	16h	8	74	312	0.567
75	16h	11	78	64	0.610
	16h	12	87	65	0.533
	16h	13	64	183	1.234
750	16h	16	69	117	0.335
	16h	17	58	155	0.820
	16h	18	56	163	0.510
100 (2AAF)	16h	21	66	60	0.357
	16h	22	82	126	0.322
	16h	23	85	40	0.473

1 = Viability determined by means of trypan blue dye exclusion assay

2 = Two additional cultures were initiated for each animal in order to examine the attachment of the cells using the neutral red absorption assay (NR-assay, 2). After an attachment period of 1.5 h the cells were washed and refed with medium containing neutral red (50 µg/ml). After an incubation period of 3 h the cultures were rinsed and the incorporated dye eluted with 50% ethanol supplemented with 1% acetic acid. The values represent the mean of two independent cultures.

TABLES OF RESULTS

INDIVIDUAL ANIMALS

Test article: LGH 10183/1

SUMMARY OF THE RESULTS

Treatment	Grains per nucleus			Grains per cytoplasm area			Net grains per nucleus		
	Mean *		Standard deviation	Mean *		Standard deviation	Mean *		Standard deviation
Solvent control 1. Animal : a.bid. /16h	4.51	±	2.49	8.04	±	2.98	-3.53	±	3.24
Solvent control 2. Animal : a.bid. /16h	4.01	±	2.32	6.36	±	2.38	-2.35	±	2.83
Solvent control 3. Animal : a.bid. /16h	6.58	±	4.75	9.60	±	5.94	-3.02	±	4.84
Positive control 1. Animal: 2AAF (100.00 mg/kg/16h)	24.84	±	11.90	4.87	±	2.50	19.97	±	11.79
Positive control 2. Animal: 2AAF (100.00 mg/kg/16h)	21.89	±	9.18	4.63	±	2.13	17.26	±	9.24
Positive control 3. Animal: 2AAF (100.00 mg/kg/16h)	17.96	±	10.10	6.54	±	3.14	11.42	±	9.30
1. Dose level 1. Animal (75.00 mg/kg/16h)	3.42	±	2.30	6.33	±	2.33	-2.91	±	2.79
1. Dose level 2. Animal (75.00 mg/kg/16h)	3.91	±	2.27	6.30	±	2.94	-2.39	±	3.22
1. Dose level 3. Animal (75.00 mg/kg/16h)	3.63	±	2.08	5.79	±	2.37	-2.16	±	2.41
2. Dose level 1. Animal (750.00 mg/kg/ 4h)	3.66	±	1.91	6.58	±	2.14	-2.92	±	1.94
2. Dose level 2. Animal (750.00 mg/kg/ 4h)	4.02	±	1.82	6.97	±	2.56	-2.95	±	2.41
2. Dose level 3. Animal (750.00 mg/kg/ 4h)	4.55	±	2.68	8.60	±	3.35	-4.05	±	3.26
2. Dose level 1. Animal (750.00 mg/kg/16h)	3.76	±	2.35	6.10	±	2.58	-2.34	±	2.82
2. Dose level 2. Animal (750.00 mg/kg/16h)	4.51	±	2.80	8.52	±	2.89	-4.01	±	3.11
2. Dose level 3. Animal (750.00 mg/kg/16h)	3.68	±	2.23	5.39	±	2.37	-1.71	±	2.55

* Mean of 100 cells

Test article: LGH 10183/1

NUCLEAR GRAIN COUNT DISTRIBUTION

Treatment			Grain count per cent:												
			> 0	> 1	> 5	> 10	> 20	> 30	> 40	> 50	> 60	> 70	> 80	> 90	> 100
Solvent control : a.bid. /16h	Mean*:	4.51	100	94.0	29.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control : a.bid. /16h	Mean*:	4.01	100	87.0	25.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control : a.bid. /16h	Mean*:	6.58	100	90.0	54.0	16.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Positive control: 2AAF (100.00 mg/kg/16h)	Mean*:	24.84	100	100	99.0	91.0	58.0	27.0	12.0	4.0	1.0	0.0	0.0	0.0	0.0
Positive control: 2AAF (100.00 mg/kg/16h)	Mean*:	21.89	100	100	98.0	89.0	54.0	15.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
Positive control: 2AAF (100.00 mg/kg/16h)	Mean*:	17.96	100	100	96.0	74.0	35.0	16.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
1. Dose level (75.00 mg/kg/16h)	Mean*:	3.42	100	81.0	19.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1. Dose level (75.00 mg/kg/16h)	Mean*:	3.91	100	89.0	18.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1. Dose level (75.00 mg/kg/16h)	Mean*:	3.63	100	82.0	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/ 4h)	Mean*:	3.66	100	92.0	13.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/ 4h)	Mean*:	4.02	100	98.0	16.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/ 4h)	Mean*:	4.55	100	100	26.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/16h)	Mean*:	3.76	100	85.0	19.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/16h)	Mean*:	4.51	100	88.0	30.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/16h)	Mean*:	3.68	100	80.0	21.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Mean of 100 cells

TABLES OF RESULTS

DOSE GROUPS

Test article: LGH 10183/1

SUMMARY OF THE RESULTS

Treatment	Grains per nucleus		Grains per cytoplasm area		Net grains per nucleus	
	Mean*	Standard deviation	Mean*	Standard deviation	Mean*	Standard deviation
Solvent control : a.bid. /16h	5.03 ±	3.54	8.00 ±	4.27	-2.97 ±	3.76
Positive control: 2AAF (100.00 mg/kg/16h)	21.56 ±	10.80	5.35 ±	2.75	16.22 ±	10.76
1. Dose level (75.00 mg/kg/16h)	3.65 ±	2.22	6.14 ±	2.56	-2.49 ±	2.83
2. Dose level (750.00 mg/kg/ 4h)	4.08 ±	2.19	7.38 ±	2.86	-3.31 ±	2.64
2. Dose level (750.00 mg/kg/16h)	3.98 ±	2.49	6.67 ±	2.94	-2.69 ±	2.99

* Mean of 3 animals, 100 cells each

Test article: LGH 10183/1

NUCLEAR GRAIN COUNT DISTRIBUTION

Treatment			Grain count per cent:											
			> 0	> 1	> 5	> 10	> 20	> 30	> 40	> 50	> 60	> 70	> 80	> 90	> 100
Solvent control : a.bid. /16h	Mean*:	5.03	100	90.3	36.0	6.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Positive control: 2AAF (100.00 mg/kg/16h)	Mean*:	21.56	100	100	97.7	84.7	49.0	19.3	6.0	1.3	0.3	0.0	0.0	0.0	0.0
1. Dose level (75.00 mg/kg/16h)	Mean*:	3.65	100	84.0	17.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/ 4h)	Mean*:	4.08	100	96.7	18.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Dose level (750.00 mg/kg/16h)	Mean*:	3.98	100	84.3	23.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Mean of 3 animals, 100 cells each

CONCLUSIONS

The test article LGH 10183/1 was assessed in the in vivo/ in vitro UDS assay for its potential to induce DNA repair (UDS) in the hepatocytes of rats.

The test article was formulated in aqua bidest.. This suspending agent was used as negative control. The volume administered orally was 10 ml/kg body weight (b.w.). After a treatment period of 4 and 16 hours, respectively, the animals were narcotized and sacrificed by liver perfusion. Primary hepatocyte cultures were established and exposed for 4 hours to ^{3}HTdR which is incorporated if UDS occurs (3).

The test article was tested at the following dose levels:

4 hour treatment period:		750 mg/kg b.w.
16 hour treatment period:	75 and	750 mg/kg b.w.

For each dose level, including the controls, hepatocytes from three treated animals were assessed for the occurrence of UDS.

No toxic reactions of the animals occured at any of the treatment periods or dose groups. However, the viabilities of hepatocytes isolated from 2 out of 3 animals treated for 16 h with 750 mg/kg b.w. were slightly decreased. The in vitro attachment of the hepatocytes was not dramatically affected due to the in vivo pretreatment with the test article. The interindividual variations obtained for the numbers of isolated hepatocytes as well as for the attachment-efficiency (NR-assay) are in the range of our historical laboratory control. In case of hepatotoxicity the NR-values expected would be approximately zero.

No dose level of the test article revealed UDS induction in the hepatocytes of the treated animals as compared to the current negative controls. Neither the nuclear grains nor the resulting net grains were enhanced due to the in vivo treatment of the animals with the test article for 4 hours or 16 hours, respectively.

An appropriate reference mutagen (2-AAF, 100 mg/kg b.w.) was used as positive control. In vivo treatment with 2-AAF revealed distinct increases in the number of nuclear and net grain counts.

In conclusion, it can be stated that during the described study and under the experimental conditions reported, the test article did not induce DNA-damage leading to repair synthesis in the hepatocytes of the treated rats.

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