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Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing

A Bibliography with Abstracts

To Assist In:

- Refining Existing Test Methods
- Reducing Animal Usage
- Replacing Animals As Test Systems

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The Scientific Community, concerned about animal welfare, is sensitive to concerns regarding how and why animals are used in biomedical research and testing to evaluate the toxicological potential of various substances. Although alternatives to methods based on the use of animals may not satisfy all requirements and needs of the biomedical research and toxicologic testing communities, alternatives to the use of vertebrates are being developed and evaluated. Research on such methodologies is aimed at refining procedures to reduce pain and discomfort; reduce the number of animals required to provide scientifically valuable results; and to replace live vertebrates when an alternative methodology can be verified and validated by the scientific community.

The purpose of these bibliographies on "animal alternatives" is to provide a survey of the literature in a format which facilitates easy scanning. This bibliography includes citations from published articles, books, book chapters, and technical reports. Citations to items in non-English languages are indicated with [ ] around the title. The language is also indicated. Citations with abstracts or annotations relating to the method are organized under subject categories. This publication features citations which deal with methods, tests, assays or procedures which may prove useful in establishing alternatives to the use of intact vertebrates. Citations are selected and compiled through searching various computerized on-line bibliographic databases of the National Library of Medicine, National Institutes of Health.

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Suggestions and comments are welcome.

ALTERNATIVES TO ANIMALS
0
Hitchman N, Leaver M, George S. ALTERNATIVES TO WHOLE ANIMAL TESTING USE OF CDNA PROBES FOR STUDIES OF PHASE I AND II ENZYME INDUCTION IN ISOLATED PLAICE HEPATOCYTES. Seventh International Symposium on Responses of Marine Organisms to Pollutants (Primo 7), Goteborg, Sweden, April 20-22, 1993. Marine Environ Res 1994;39(1-4):289-92.

1
Reinhardt CA. THE SIAT RESEARCH TEACHING AND CONSULTING PROGRAM IN THE AREA OF IN VITRO TOXICOLOGY EXPERIMENTAL RESEARCH SCREENING AND VALIDATION. In: Reinhardt CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress; Symposium; 1992 Nov 30; Zurich, Switzerland. New York: VCH Publishers, Inc: 1994. p. 89-98.

2
Spielmann H. HET-CAM TEST. Methods Mol Biol 1995;43:199-204.

3
Atterwill CK. ALTERNATIVE METHOD OF ASSESSING TOXICITY. Methods Mol Biol 1995;43:1-9.

4
Alarie Y, Nielsen GD, Andonian-Haftvan J, Abraham MH. PHYSICOCHEMICAL PROPERTIES OF NONREACTIVE VOLATILE ORGANIC CHEMICALS TO ESTIMATE RD50: ALTERNATIVES TO ANIMAL STUDIES. Toxicol Appl Pharmacol 1995;34(1):92-9.

This article presents the correlations obtained between the results on the potency of nonreactive airborne chemicals as sensory irritants and several of their physicochemical properties. The potency of airborne sensory irritants obtained from a reflexively induced decrease in respiratory frequency has been measured in the past using mice. Typically, their potency has been expressed as the exposure concentration necessary to decrease respiratory frequency by 50% (RD50). A large database of RD50 values is now available and such values are highly correlated with occupational exposure guidelines such as threshold limit values (TLVs). We used the nonreactive volatile organic chemicals from this database, for which relevant physicochemical variables are available or can be calculated. These variables were vapor pressure (P) or Ostwald gas-liquid partition coefficients (L). The liquids used for L values were n-hexadecane, octanol, N-formylmorpholine, tri-(2-ethylhexyl)phosphate, and olive oil. Excellent correlations were found between log RD50 and log P, as well as between log RD50 and log L16, log L(Oct), log L(NFM), log L(EHP), or log L(Oil). It follows that as an alternative to the bioassay, these physicochemical variables can be used to estimate RD50 of nonreactive volatile organic chemicals. Appropriate exceptions to general estimation of RD50 values from physicochemical variables are also presented, as well as the most appropriate estimates which can be obtained within homologous series.

5
Ehrich M. USING NEUROBLASTOMA CELL LINES TO ADDRESS DIFFERENTIAL SPECIFICITY

that these cells still had a tendency to form cobblestone-like cells and cell tracks, but to a substantially lower degree. The present results support the hypothesis that HA-1A suppresses the proliferative and morphological effects of endotoxin on rat middle ear epithelium and may play an important role in the pathogenesis of otitis media.

35
Muraki Y, Yamada M, Nii S, Kumon H, Ohmori H. [IN VITRO ASSESSMENT OF RELATIVE PHOTOTOXICITY OF QUINOLONE ANTIBACTERIAL AGENTS BY REDUCTION OF NEUTRAL RED UPTAKE IN CULTURED CELLS.] Nippon Kagaku Ryoho Gakkai Zasshi 1995;43(3):357-60. (Jpn)

The relative phototoxicity of 10 antibacterial drugs in the quinolone group was detd. by an in vitro assay, in which redn. of neutral red uptake was used as a marker of cell injury. Human embryonal lung fibroblasts or Vero cells derived from a green monkey kidney were incubated with potential phototoxins. The cell cultures were irradiated with long wave-length UV, and the capacity for neutral red uptake was detd. The phototoxicity of 10 quinolones was much lower than that of doxycycline, a known photosensitizer. Among them, enoxacin, lomefloxacin, ciprofloxacin, ofloxacin, and nalidixic acid demonstrated higher phototoxicity, suggesting a good correlation with the clin. occurrence of photosensitivity. Norfloxacin, balofloxacin (Q-35), AM-1155, and T-3761 had less potent phototoxicity. This methodol. may provide a useful rapid method to quantitate the phototoxic potential of newly developed drugs.

36
Dumitrescu M, Jucu V, Zaharia CN, Belu O, Rojanschi D, Diaconu C, Talos D, Panaitescu M. [ACTION OF SOME AMPHIPHILIC DRUGS ON HUMAN FIBROBLASTS IN VITRO.
POSSIBLE ANTIVIRAL AND ANTITUMOR USES.] Rev Roum Virol 1993;44(3-4):211-21. (Fre)

The release of lactic dehydrogenase (LDH) from human embryo fibroblasts in vitro was used as a test of membrane stability. Two amphiphilic drugs, metomidate and timolol, stabilized the membrane, as shown by a reduced release of LDH. Another drug, Ca dobesylate, had the opposite effect, making the membrane less stable. The use of the LDH test for the selection of some natural complexes or synthetic drugs with membrane-stabilizing and hence potential antiviral activity (by inhibiting virus penetration into cells) is proposed. Furthermore, LDH activity was inhibited by metomidate, causing the intracellular accumulation of lactate and consequent lowering of intracellular pH. The use of metomidate for potentiating the action of classical antitumor drugs, lowering tumor cell pH, is proposed.

37
O'Hare S, Atterwill CK, editors. METHODS IN MOLECULAR BIOLOGY. Vol 43, IN VITRO TOXICITY TESTING PROTOCOLS. Totowa (NJ): Humana Press Inc; 1995. 332 p.

38
Langner A, Melzig MF, Kempa S, Krause A. [THE USE OF LYMPHOCYTE CULTURES FOR

considerable binding of these compounds to macromolecules. BHA and TBHQ, as well as TBQ, induced a dose-dependent increase in cell proliferation of phytohaemagglutinin-stimulated lymphocytes, 50 muM being the optimal dose. Since BHA is metabolized to TBHQ, it is not clear which compound is responsible for the proliferation enhancing effects observed in culture. Inhibition of TBHQ metabolism to its semiquinone radical by acetylsalicylic acid (ASA) reduced the increase in labelling indices induced by TBHQ. This indicates that this metabolic pathway is involved in the enhancement of cell proliferation induced by the hydroquinone. HPLC-ECD analysis of oxidative DNA damage in lymphocytes exposed to 10, 50 and 100 muM BHA, TBHQ or TBQ respectively showed that BHA was not capable of inducing oxidative DNA damage to a significant degree. TBQ and, in particular, TBHQ at a dose of 50 muM (the optimal dose for induction of cell proliferation), however, increased lymphocyte 7-hydroxy-8-oxo-2'-deoxyguanosine formation by 320 and 680% respectively. Inhibition of prostaglandin H synthase by ASA in cultures treated with TBHQ decreased the oxidation ratio significantly, confirming the significance of this enzyme system in the mechanism of toxicity of BHA.

48
Hilton J, Kimber I. THE MURINE LOCAL LYMPH NODE ASSAY. Methods Mol Biol 1995;43:227-35.

49
Gillies GE, Buckingham JC. THE APPLICATION OF IN VITRO MODELS OF HYPOTHALAMIC FUNCTION IN TOXICITY TESTING. Methods Mol Biol 1995;43: 95-107.

50
Gillies GE, Buckingham JC. THE APPLICATION OF IN VITRO MODELS OF ANTERIOR PITUITARY FUNCTION IN TOXICITY TESTING. Methods Mol Biol 1995;43:81-93.

51
Hodgson E. CHEMICAL AND ENVIRONMENTAL FACTORS AFFECTING METABOLISM OF XENOBIOTICS. In: Hodgson E and Levi PE, editors. Introduction to Biochemical Toxicology. 2nd ed. East Norwalk (CT): Appleton and Lange; 1994. p. 153-75.

52
Jiang Y, Moller G. IN VITRO EFFECTS OF HGCl2 ON MURINE LYMPHOCYTES: I. PREFERABLE ACTIVATION OF CD4+ T CELLS IN A RESPONDER STRAIN. J Immunol 1995;154(7):3138-46.

Mercury-induced autoimmune disorders have been demonstrated in rats and mice injected with HgCl2. We have studied the ability of HgCl2 to activate murine lymphocytes in vitro and found that it induced increased DNA synthesis, which peaked at days 4 to 6. Other metal ions, such as Mg2+ and Zn2+, had no or much less effect. Consistent with the in vivo studies, there were strain differences, and the most significant increase in thymidine uptake was induced in A.SW and BALB/c spleen cells. Both T lymphocytes and adherent cells were required for activation, and anti-CD4 Ab completely abrogated HgCl2-induced proliferation, suggesting the involvement of T CD4+ and CD8+ T cells from BALB/c mice (responder strain). In contrast, only CD8+ T cells from the nonresponder DBA/2 mice were transformed. These findings indicate that helper

T cells play a crucial role for the immunologic effects caused by HgCl2 and determine the ability of different mouse strains to respond to HgCl2.

53
Hwang DF, Lin JF, Jengs SS. STUDIES ON SUSPENDING CONDITIONS FOR ISOLATED EEL HEPATOCYTES. J Fish Soc Taiwan 1994;21(3):273-80.

To establish fish hepatocytes as experimental materials for in vitro system of toxicological and metabolic studies, the livers of Japanese eel Auguilla japonica were used to isolate hepatocytes by using perfusion method. Based on the viability of hepatocytes by staining with trypan blue the optimal suspending conditions for eel hepatocytes were determined. It was found that the optimal suspending temperature, time, cell concentration and pH value were 37~ C, 2 hr, 207 cells/ml and 7.5, respectively. It was also found that the viability of isolated eel hepatocytes was improved when the suspending buffer was added with either 5.6 mM glucose or 2.5 mM Ca++.

54
Kristen U, Kappler R. THE POLLEN TUBE GROWTH TEST. Methods Mol Biol 1995;43:189-98.

55
Blein-Sella O, Adolphe M. RABBIT ARTICULAR CHONDROCYTE FUNCTIONAL TOXICITY TEST. Methods Mol Biol 1995;43:169-75.

56
Bidey SP. THYROID FOLLICULAR CELLS IN MONOLAYER CULTURE IN VITRO MODELS FOR THYROID TOXICITY TESTING. Methods Mol Biol 1995;43:33-42.

57
Becerro MA, Uriz MJ, Turon X. MEASURING TOXICITY IN MARINE ENVIRONMENTS: CRITICAL APPRAISAL OF THREE COMMONLY USED METHODS. Experientia 1995;51(4):414-8.

Toxicity quantification is important in environmental monitoring, in the field of natural products, and in chemical ecology. The sensitivity and precision of three commonly used methods detecting toxicity in marine environments were compared, using the toxic marine sponge Crambe crambe as a test organism. The paper disk diffusion method (run with marine bacteria) showed the least sensitivity and did not permit toxicity levels to be quantified. The sea urchin and the MICROTOX tests showed greater sensitivity, and the latter had the higher precision. The relative performance of these methods is discussed. It is concluded that the MICROTOX bioassay displays the best characteristics for toxicity quantification.

58
Blanquart C, Giuliani I, Houcine O, Jeulin C, Guennou C, Marano F. IN VITRO EXPOSURE OF RABBIT TRACHEAL EPITHELIUM TO SO2: EFFECTS ON MORPHOLOGY AND
CILIARY BEATING. Toxicol In Vitro 1995;9(2):123-32.

1995;10(3):203-8.

The cytokinesis-block micronucleus assay was used to measure radiosensitivity in vitro in a panel of seven cell lines. Six of these cell lines were used to study the major parameters of this assay. We observed varying sensitivities following cytochalasin-B exposure. Treatment with 1 mug/ml cytochalasin-B for 24 h reduced cell survival in four of the six cell lines by > 60%. Cytochalasin-B concentration and post-irradiation culture time were both found to influence cell-response. In three cell lines (V39, V134 and HX142), a decrease in cytochalasin-B concentration (2-0.5 mug/ml) resulted in an increase in the frequency of radiation-induced micronuclei per binucleate cell. In other cell lines, either the opposite (V7M, CHO-K1) or no effect (WiDr) was seen. A linear dose-response was observed between induced damage expressed as the frequency of micronuclei and radiation dose in all but one melanoma (V39) cell line. Evidence for radiation-induced division-delay, with the maximum frequency of binucleation in irradiated cultures occurring 24-48

h after that of controls, was only seen in two cell lines. Of particular note, and in contrast to some other published reports, was the lack of a general correlation between cell-response measured in the clonogenic and the cytokinesis-block micronucleus assays. Consideration of lethal lesions, determined from the clonogenic dose-response curve, with respect to micronucleus frequency showed a complex relationship, with one micronucleus per binucleate cell corresponding to a wide range of lethal lesions depending on the cell line. It has been postulated that the binucleate cell with no micronuclei may represent the surviving cell; however, we found no correlation between the slope of the frequency of these cells with respect to radiation dose and the clonogenic alpha slope. These observations should be considered prior to attempting to use the cytokinesis-block micronucleus assay to measure in vitro radiosensitivity in human tumour cells.

64
Clare C. MUTATION ASSAYS IN BACTERIA. Methods Mol Biol 1995;43:297-306.

65
Dean S. MEASUREMENT OF UNSCHEDULED DNA SYNTHESIS IN VITRO USING PRIMARY RAT
HEPATOCYTE CULTURES. Methods Mol Biol 1995;43: 267-76.

66
Bryan FL. PROCEDURES TO USE DURING OUTBREAKS OF FOOD-BORNE DISEASE. In: Murray
PR, et al, editors. Manual of Clinical Microbiology. 6th ed. Washington DC:
American Society for Microbiology; 1995: p. 209-26.

67
Edwards AJ, Anderson D, Phillips BJ. INDUCTION OF POLYPLOIDY IN HUMAN LYMPHOCYTES IN VITRO BY EXCESS ADENINE, BUT NOT BY ADENOSINE. Environ Mol Mutagen 1995;25(3):197-201.

It is known that high levels of DNA precursors can be both clastogenic and

mutagenic in cultured cell lines and in vivo. The purpose of the present study was to examine at an observational level the cytogenetic effects of adenine and adenosine in primary human cell cultures. Human peripheral blood lymphocytes from four donors were cultured and treated with a range of concentrations of adenine and adenosine. Although no increase in sister chromatid exchange (SCE) frequency was observed with either compound, there was a statistically significant, dose-related increase in the proportion of polyploid cells in cultures treated with adenine, but not in those treated with adenosine. Some of the polyploid metaphases found after adenine treatment contained diplochromosomes, suggesting that endoreduplicotion might have been involved in polyploid formation in these cells. It is concluded that a high level of adenine can cause genetic changes in human lymphocytes by interfering with mitosis, perhaps by disturbing the balance of DNA precursor pools.

68
Dinjus U, Haenel I. CULTIVATION OF SALMONELLA IN CONTACT WITH EPITHELIAL CELLS. Microbiol Res 1995;150(1):99-102.

An in vitro cultivation model for Salmonella having contact to epithelial cells was developed, which led to an increase in the production of toxic substances. The toxin assay on CHO-K1 cells was used for the determination of the toxic activities. Salmonella strains cultivated in contact with a monolayer of the intestinal cell line IEC-6 produced considerably more toxin than Salmonella strains cultivated on VERO cells. The toxin formed was heat-labile.

69
Falk PM, Sabater RT, Carballo DD Jr. RESPONSE OF THE HUMAN HEMATIC TISSUE CULTURES HEP-G2 AND WRL-68 TO COCAINE. J Pharmacol Toxicol Methods 1995;33(2):113-20.

The hydrolytic metabolism of cocaine into benzoylecgonine, ecgonine methyl ester, and ecgonine was studied in the human hepatoma cell line Hep-G2 and in the nontumorigenic fetal hepatic cell line WRL-68. Also, the toxicological response of these cells to cocaine was compared to previously published results obtained with perfused liver cells and in vivo systems. Our experiments indicated that Hep-G2 appear to have similar metabolic and toxicological patterns to in vivo and perfused cell systems. The WRL-68 tissue culture system was found to be less similar. These results suggest Hep-G2 cells can be utilized to study cocaine metabolism and toxicology, and possibly in studies involving other xenobiotic compounds.

70
Fiskesjo G. ALLIUM TEST. Methods Mol Biol 1995;43:119-27.

71
Male KB, Brown RS, Luong J HT. ENZYMATIC OXIDATION OF WATER-SOLUBLE CYCLODEXTRIN-POLYNUCLEAR AROMATIC HYDROCARBON INCLUSION COMPLEXES, USING
LIGNIN PEROXIDASE. Enzyme Microb Technol 1995;17(7):607-14.

alpha-, beta-, gamma-, and 2-hydroxypropyl-beta-cyclodextrins were capable of forming water-soluble inclusion complexes with several polynuclear aromatic hydrocarbons (PAHs). The highest solubilities were noted for beta-cyclodextrin and 2-hydroxypropyl-beta-cyclodextrin (hpbetaCD). The solubility of PAHs in hpbetaCD was enhanced 224-fold and 7,500fold for naphthalene and benzo(a)pyrene, respectively, with other PAHs yielding values between these limits. The ability of lignin peroxidase (LiP) to cyclodextrin-included substrates was similar to that previously reported for mixed solvent systems. The enzyme oxidized anthracene, pyrene, and benzo(a)pyrene but not naphthalene, phenanthrene, chrysene, and benzo(e)pyrene. The lignin peroxidase exhibited a preference for oxidizing either anthracene or benzo(a)pyrene when mixed with pyrene. On the basis of fluorescence measurement, anthracene and benzo(a)pyrene were easily distinguished by exciting at 250 nm for anthracene and 295 nm for benzo(a)pyrene. Veratryl alcohol severely inhibited the pyrene assay, with 50% inhibition noted at 0.3 mm while veratryl alcohol activated the reactions between LiP and either anthracene or benzo(a)pyrene. Maximal activation was obtained at 1.5 mm veratryl alcohol and no inhibitory effect was detected up to 4.0 mm. Under identical conditions, the rate of reaction with veratryl alcohol (4.0 mm) was 11- and 14-fold faster for benzo(a)pyrene and anthracene, respectively, when compared to the assays in the absence of veratryl alcohol.

72
Fisher RL, Hasal SJ, Sipes IG, Gandolfi AJ, Brendel K. COMPARATIVE METABOLISM AND TOXICITY OF DICHLOROBENZENES IN SPRAGUE-DAWLEY, FISCHER-344 AND HUMAN
LIVER SLICES. Hum Exp Toxicol 1995;14(5):414-21.

1 Precision-cut liver slices, prepared from Sprague-Dawley and Fischer-344 rats and donated human liver tissue, were used to identify differences in 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB) and 1,4-dichlorobenzene (1,4-DCB) metabolism and how it may relate to toxicity. 2 Rat and human liver slices were incubated with 1 mM of either dichlorobenzene to determine metabolism and toxicity, at 2 and 6 h of organ culture. 3 The human liver slices metabolised the dichlorobenzenes to a greater extent than those from either of the rat strains. Liver slices from the Fischer-344 strain had a higher metabolic rate than the slices from the Sprague-Dawley rat strain. 4 The metabolic rate of dichlorobenzene isomers did not consistently correlate with its toxicity. For example, human slices did not exhibit any hepatotoxicity, even though they metabolised these compounds to a greater extent than either rat strain. 5 Cross species covalent binding did not correlate with toxicity endpoints measured in this study. 6 The phase two metabolite profiles for each of the isomers in human and rat slices were similar in that the glutathione-cysteine conjugate was the major metabolite. 7 The use of an in vitro system which utilises human liver slices might provide an important bridge between animal derived data and the human situation.

73
Na MR, Koo SK, Kim DY, Park SD, Rhee SK, Kang KW, Joe CO. IN VITRO INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BY CHEMICAL CARCINOGENS. Toxicology 1995;98(1-3):199-206.

This study was conducted to assess the effects of chemical carcinogens on the gap junction-mediated intercellular communication in cultured mammalian cells. The method of scrape-loading dye transfer of lucifer yellow was adapted as a measure of gap junctional communication. Clone 9 cells derived from rat liver were treated with a model chemical carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and the gap junctional communication was assessed by measuring the transfer of scrape-loaded lucifer yellow dye. When cells were treated with the carcinogen at 0.3 mg/ml, the fluorescent dye transfer was inhibited by 90% in 60 min. Other chemical agents, which include direct or indirect carcinogens and antitumor drugs, were also examined for their effects on the gap junctional communication. Direct carcinogens, such as MNNG, hydroxylamine and ethidium bromide, exhibited strong inhibition of intercellular communication, while indirect carcinogens, such as aflatoxin B1 and ethionine, exerted minor effects. Effects of test chemicals on the cell communication through gap junctions were readily quantitated by counting the number of cells stained with the fluorescent dye.

74
Naughton BA, Sibanda B, San Roman J, Naughton GK. CHARACTERIZATION AND USE OF LONG-TERM LIVER CULTURES TO EVALUATE THE TOXICITY OF CYCLOPHOSPHAMIDE OR
BENZENE TO BONE MARROW CULTURES. In: Reinhardt CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress; Symposium; 1992 Nov 30; Zurich, Switzerland. New York: VCH Publishers, Inc: 1994. p. 147-57.

Flint O. A TIMETABLE FOR REPLACING REDUCING AND REFINING ANIMAL USE WITH THE HELP OF IN VITRO TESTS THE LIMULUS AMOEBOCYTE LYSATE TEST LAL AS AN EXAMPLE.
In: Reinhardt, CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress; Symposium; 1992 Nov 30; Zurich, Switzerland. New York: VCH Publishers, Inc; 1994: p. 27-43.

75
Berg OH, Henriksen RN, Steinsvag SK. THE EFFECT OF A BENZALKONIUM CHLORIDE-CONTAINING NASAL SPRAY ON HUMAN RESPIRATORY MUCOSA IN VITRO AS A
FUNCTION OF CONCENTRATION AND TIME OF ACTION. Pharmacol Toxicol 1995;76(4):245-9.

Human respiratory mucosa was exposed to oxymetazoline nasal spray in varying concentrations and for varying periods of time in vitro. The drug destroyed the tissue in a concentration- and time-dependent manner. In the experiments with various concentrations of the spray, some tissue fragments retained their viability throughout the experiment. This number increased parallel to a decrease in concentrations of the test substance. All the tissue fragments exposed to undiluted nose spray underwent severe destructive alterations during the exposure period. These alterations appeared first and were most extensive in those exposed for the longest periods of time. It has previously

2 to 3 days. Activation of lymphocytes and assessment of tumoricidal function by a chromium release assay were performed directly in a standard control medium (RPMI 1640 containing 2 mM glutamine, 100 micrograms/ml streptomycin, 100 units penicillin, 5% heat-inactivated human AB serum, and 5 mM 4-(2-hydroxyethyl)-1-piperazinesulfonic acid) and in 50% ascitic fluid (50% by volume filter-sterilized ascites with 50% of the above-mentioned control medium). Target cells were added directly into the medium in which the lymphocytes were activated in order to more closely mimic in vivo conditions. Lymphocytes, activated by IL-12 in 50% ascitic fluid, were able to lyse autologous tumor cells in 3 of 6 assays and were able to lyse SKOV3 cells (an ovarian cancer cell line) in 5 of 7 assays. The results were not significantly different in the control medium. When both IL-2 and IL-12 were used to activate lymphocytes in 50% ascitic fluid, significant cytotoxicity was generated in 6 of 6 autologous assays and in all 7 patient assays using SKOV3 as a target ($P < 0.05$). Synergy between the two cytokines was seen in all 13 patient assays in ascitic medium compared to only 5 of 13 assays in control medium. Additionally, when lymphocytes were stimulated with both IL-2 and IL-12, significantly greater cytotoxicity was seen in the ascitic fluid medium compared to the control medium in 13 of 14 assays ($P < 0.05$). No significant tumoricidal activity was seen by lymphocytes maintained in either medium without the addition of IL-2 or IL-12. Ascitic fluid consistently potentiates the synergy between IL-2 and IL-12 in generating cytotoxicity against ovarian cancer cells but does not increase cytotoxicity induced by IL-12 alone. IL-12 by itself activates tumoricidal activity of lymphocytes in ascitic fluid; however, the addition of IL-2 increases the degree and consistency of this effect. These data support the possibility that IL-12 may warrant further investigation as a potential therapeutic agent in the treatment of advanced ovarian cancer.

112
Kaspers GJ, Veerman AJ, Pieters R, Van Zantwijk I, Hahlen K, Van Wering ER. DRUG COMBINATION TESTING IN ACUTE LYMPHOBLASTIC LEUKEMIA USING THE MTT ASSAY. Leuk Res 1995;19(3):175-81.

Drug resistance assays may be useful to identify drug interactions. For this purpose, we studied three drug combinations, each at 8-12 concentrations, with the MTT assay in acute lymphoblastic leukemia (ALL) samples from 34 children btained at initial diagnosis. This resulted in a total of 518 comparisons between expected and observed leukemic cell survivals. The combinations prednisolone (PRD) with vincristine (VCR), PRD with mafosfamide (MAF), and PRD with daunorubicin (DNR) were tested without technical difficulties, and without an increased assay variation as compared to single drugs. We observed a marked heterogeneity in drug interactions between patients, between combinations, and between different concentrations within one specific combination. Between PRD+VCR, synergism was found in 46%, antagonism in 18%, and additivity in 36% of the 228 observations. Between PRD+MAF, synergism was found in 51%, antagonism in 20%, and additivity in 29% of the 140 observations. Between PRD+DNR, synergism was found in 35%, antagonism in 31%, and additivity in 34% of the 150 observations. PRD+VCR and PRD+MAF showed more often synergism than PRD+DNR, while antagonism was observed more frequently between PRD+DNR ($p < 0.05$). However, the magnitude of antagonism