

Host-Parasite Relationships in Echinococcosis: *

X. Laboratory Evaluation of Chemical Scolicides as Adjuncts to Hydatid Surgery

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PRIOR to surgical removal of a hydatid cyst it is common practice for the surgeon to withdraw a portion of the cyst fluid by syringe and to replace it with a presumably scolicidal solution. The intent is to kill scolices and germinal tissues thereby eliminating risk of secondary cyst formation through spillage of viable hydatid sand into the body cavity of the patient. Formalin has been most commonly used for this purpose. Iodine preparations, corrosive sublimate, phenol and other chemicals also have been suggested² although laboratory evidence is not available upon which to assess their comparative efficacies. In the present study, therefore, a number of chemicals, including some of the newer germicides, have been compared for *in vitro* scolicidal activity and the more active of these have been tested for toxicity to white mice upon intraperitoneal injection.

Materials and Methods

Hydatid cysts of the liver and lungs of cattle, sheep and goats were obtained from local abattoirs within a few hours of

slaughter of their hosts. Viable scolices were collected and washed three times in physiological saline solution by suspension and sedimentation.

Two ml. of scolicide solution, prepared in physiological saline, were placed in a 100 × 12 mm. round bottom test tube. Tubes were partially immersed in a 37° C. water bath and, following temperature equilibration, a drop of settled scolex suspension (containing approximately 500 scolices) was carefully added to each, and gently but thoroughly mixed with the scolicide solution. Control tubes were prepared using scolices and saline alone. Following 10, five or one minutes exposure, the scolices were washed four times in physiological saline by suspension and slow centrifugation, resuspended in 1.0 ml. of saline and returned to the water bath.

After 30 minutes at 37° C. they were examined microscopically for viability. For this purpose two to three drops of suspension were placed on each of two glass slides on a warm stage. The first slide preparation was examined for scolex motility and one drop of a 1:1,000 solution of janus green or eosin was added to the second.^{1, 3, 5} Living scolices did not take these stains while those gently heat-killed at 60° C or killed chemically stained uniformly dark in one to five minutes. This technic proved

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quite satisfactory and it was not found necessary to resort to the chemical stimulation method of Mayer⁴ nor to a search for active flame cells.⁷ Perez-Fontana⁵ was able to distinguish normal from abnormal (dead?) scolices by the fact that abnormal scolices stained very rapidly with gentian violet while the normal ones did not. However, caution must be used in interpretation since unpublished findings (Schwabe, Haddian and Koussa, *in press*) indicate that gentian violet itself affects the scolex adversely.

The initial screening of chemicals was done at 10 minutes exposure only and their approximate minimum scolicidal concentrations determined. With few exceptions, chemicals appreciably less scolicidal than formalin at the same concentration were not examined further. For those chemicals selected for further evaluation minimum scolicidal concentrations were redetermined for 10 and also for five and one-minute exposures. These latter experiments were all run in duplicate on the same *pool* of scolices. A portion of each scolex suspension after exposure to the scolicide, and of each control preparation was washed in saline and inoculated intraperitoneally into a white mouse of 44 days of age or less. Immature mice are susceptible to secondary echinococcosis by that route.⁸ These mice were killed and examined for developing cysts and dead scolex debris after 100 days.⁸

The more promising scolicides were each injected intraperitoneally at different dilutions into 25 to 75 white mice of approximately 28 Gm. each and the lethal dose for 50 per cent of the mice (LD₅₀) calculated.⁶

The chemicals examined were formalin (from formaldehyde solution: 37 to 41 per cent w/v); phenol (from crystals containing 98% C₆H₅OH minimum); cresol and soap solution (Lysol®); ethyl alcohol; glycine; sodium ethyl mercurithiosalicylate (Merthiolate®), acetic acid; hydrogen per-

oxide; sodium hypochlorite (fresh 5.26% solution prepared and assayed in the American University Hospital Pharmacy); potassium permanganate; urea; boric acid; sodium bicarbonate; quinine HCl; cetyltrimethylammonium bromide (Cetrimide®); 2-diphenylmethoxy-N,N-dimethylethylamine HCl (Benadryl® HCl); iodoacetic acid; iodophthalein sodium (Iodeikon®); W-50 (methaphenylene HCl, Warner-Lambert); iodinated glycerol (Organidin®, Wampole Laboratory); 2-[benzyl (2-dimethylaminoethyl) amino] pyridine HCl (Pyr benzamine® HCl); nonylphenoxy-polyethoxyethanol-iodine complex, 16.4 per cent solution (I-O-Teen®, Fuld Brothers); octoglycine dihydropentiodide (Burnham Soluble Iodine Co.); 6,9-diamino-2-ethoxyacridine lactate (Rivanol®); polyvinylpyrrolidone-iodine complex, 10 per cent solution (Isodine®); quinacrine HCl (Atebrine® HCl); acetylamino-3-triiodo-2,4,6-benzoate of N-methylglucamine (Vasurix 38®); 3,5-diacetamido-2,4,6-triiodobenzoic acid sodium salt (Hypaque® sodium); 3,5-diiodo-4-pyridone-N-acetic acid diethanolamine salt (Neo-tenebryl®); hexamine; 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline diphosphate (Chloroquine® diphosphate); N,N'-diacetyl 3,5-diamino-2,4,6-triiodobenzoic acid sodium and methylglucamine salt (Urografin®); 8-hydroxy-7-iodoquinoline-5-sulfonic acid and NaHCO₃ (Chiniofon®); diethylcarbamazine (Hetrazan®); suramin sodium (Germanin®); 1-piperazine carbo-thioic acid 4-methyl-5-methyl ester (mepiperazine); pyrilamine maleate (Neo-Antergan®); and iodine (as iodine, 1 part, and KI, 2 parts, dissolved in water).*

* Chemicals or information about them were kindly furnished by Professor A. Haddad, School of Pharmacy, American University of Beirut; the Surgery Department of the American University; Burnham Soluble Iodine Company; and Isodine Pharmacal Corporation. The white mice were of a strain obtained originally from the United States Naval Medical Research Unit 3, Cairo.

Results

The following chemicals were rejected as being inactive as scolicides during 10 minutes exposure at the concentrations given: 10 per cent urea, 6.0 per cent Atebrine, 75 per cent Vasurix "38," 50 per cent Hypaque, 35 per cent Neo-tenebryl, 3.0 per cent hexamine, 6.0 per cent Chloroquine diphosphate, 60 per cent Urografin, 10 per cent boric acid, 5.0 per cent sodium bicarbonate, 6.0 quinine HCl, 6.0 per cent Chiniofon, 6.0 per cent Hetrazan, 6.0 per cent Germanin, 6.0 per cent mepiperazine, 6.0 per cent iodoacetic acid and 6.0 per cent pyrilamine maleate.

Those chemicals which were scolicidal during 10 minutes exposure with their minimum scolicidal concentrations were: formalin (0.5%), phenol (0.5%), iodine (0.01%), glycerine (20%), Iodeikon (1%), Lysol (0.5%), Benadryl HCl (1.5%), ethyl alcohol (20%), Cetrimide (0.005%), W-50 (1%), Organidin (2%), Merthiolate (0.1%), Pyribenzamine HCl (3%), acetic acid (1%), I.O.Teen (0.5%), sodium hypochlorite (0.05%), hydrogen peroxide (0.1%), potassium permanganate (0.05%), octoglycine dihydropentiodide (0.05%), Rivanol (0.1%) and acriflavine (1%). Iodine (1%) was of questionable activity.

Final laboratory results on the more active scolicides are shown in Table 1. Judging by all three criteria of scolex motility, scolex staining and presence or absence of developing cysts in inoculated mice, only 25 per cent glycerine was scolicidal at one minute exposure. As there was poor correlation between the three viability criteria at one minute, it is likely that there was a residual action by certain chemicals. At five minutes, 0.5 per cent formalin, 0.005 per cent Cetrimide, 0.05 per cent octoglycine dihydropentiodide, 0.01 per cent iodine, 0.1 per cent hydrogen peroxide, 1.0 per cent I-O-Teen, and 0.05 per cent sodium hypochlorite were scolicidal. One per cent Iodeikon, 0.5 per cent phenol, 15 per

cent ethyl alcohol and 0.1 per cent Rivanol were scolicidal at 10 minutes exposure.

Of these chemicals, Cetrimide, iodine and Rivanol were the most toxic to mice and Isodine, ethyl alcohol and glycerine the least toxic. A relative scolicidal value for each chemical in terms of effective concentration, time of exposure and toxicity was calculated by dividing its LD₅₀ for mice by the product of the lowest effective concentration (C) and the minimum effective exposure time (T) (LD₅₀/CT). The value thus obtained for formalin (calculated in terms of formaldehyde) was 8.8. Chemicals with higher scolicidal values were: hydrogen peroxide 56.2, Cetrimide 52.0, iodine 25.8, octoglycine dihydropentiodide 16.9, sodium hypochlorite 13.6 and glycerine 10.6. The others were all of less value than formalin.

Discussion

To be a useful adjunct to hydatid surgery, a chemical should be scolicidal during a short exposure period and at a high dilution, it should be nontoxic to the patient and it should be relatively stable in solution. Of the chemicals examined here, six were scolicidal at higher dilutions than formalin for the same exposure time, and two of these (glycerine and hydrogen peroxide) also were less toxic than formalin in *absolute* terms upon intraperitoneal injection in mice. Taking factors of concentration, time and toxicity all into consideration, however, six chemicals appeared to have greater actual *scolicidal value* than formalin, with hydrogen peroxide and Cetrimide six times as *effective*. Only one of these six chemicals, sodium hypochlorite was assayed for stability in solution. It deteriorated significantly upon a few days' standing and would therefore need to be prepared fresh for each use.

While hydrogen peroxide, Cetrimide, iodine, octoglycine dihydropentiodide, sodium hypochlorite, and glycerine all appeared preferable to formalin by these cri-

TABLE 1. Comparative Efficacy of Hydatid Scolicidides

Chemical	Conc. (%)	Incubation Period (minutes)												Relative Scolicidal Value $\frac{LD_{50}}{CT}$			
		1				5				10							
		Motile	Takes Stain	Cysts Develop in Mice		Motile	Takes Stain	Cysts Develop in Mice		Motile	Takes Stain	Cysts Develop in Mice					
Formalin	0.5*	±	±	+	-	±	+	-	-	+	+	-	-	+	+	-	8.81 ⁺
Phenol	0.5	+	-	+	±	±	+	-	-	±	+	-	-	+	+	-	7.73
Cetrimide	0.005	±	±	-	-	±	+	-	-	±	+	-	-	±	+	-	1.30
Octoglycine	0.05	±	-	-	-	±	+	-	-	±	+	-	-	±	+	-	4.23
Iodine (KI)	0.01	-	+	+	-	±	+	+	-	±	+	+	-	±	+	-	1.29
Ethyl alcohol	15	+	-	+	-	±	+	+	-	±	+	+	-	±	+	-	184.9
Iodeikon	1	+	-	-	-	±	+	-	-	±	+	-	-	±	+	-	8.89
Rivanol	0.1	±	±	+	+	±	+	+	-	±	+	+	-	±	+	-	1.77
	0.05	+	-	+	-	±	+	+	-	±	+	+	-	±	+	-	231.5
Glycerine	20	±	±	-	-	±	+	-	-	±	+	-	-	±	+	-	10.6
	25	±	±	-	-	±	+	-	-	±	+	-	-	±	+	-	11.30
Isodine ⁺⁺	1	+	-	+	+	±	+	-	-	±	+	-	-	±	+	-	28.10
H ₂ O ₂	0.1	-	±	+	-	±	+	-	-	±	+	-	-	±	+	-	56.2
I-O-Teen ⁺⁺	0.5	+	-	-	-	±	+	-	-	±	+	-	-	±	+	-	4.63
	1	±	±	-	-	±	+	-	-	±	+	-	-	±	+	-	5.6
Na hypochlorite	0.005	+	-	+	+	±	+	-	-	±	+	+	-	±	+	-	3.39
	0.01	±	±	+	+	±	+	-	-	±	+	+	-	±	+	-	13.6
	0.05	-	+	+	+	±	+	-	-	±	+	-	-	±	+	-	

* Equivalent to 0.2% formaldehyde; † calculated as formaldehyde.

** mg./28Gm. (body wt.) in 96 hours.

±± Isodine and I-O-Teen were available only in solutions of the active ingredients. The LD₅₀ and $\frac{LD_{50}}{CT}$ were calculated as active ingredient.

teria, their respective LD₅₀'s for mice upon intraperitoneal injection might not accurately reflect their relative local tissue or systemic toxicities for man. It should be noted that the scolicial sodium hypochlorite solution is the same as that currently used for lavage of body cavities and organs during surgery in man. Hydrogen-peroxide solution has been used in the treatment of gastritis.

The present uncertainties in the use of scolicides, in particular the difficulty of accurately estimating the volume of the hydatid cyst *in situ*, urge upon the surgeon the employment of the most potent safe scolicide available. Until a ready means is found to determine cyst volume *in situ*, use of scolicides will remain largely guesswork in so far as the surgeon's ability to insure the injection of a safe excess of the chemical is concerned. An approach to that problem would be the incorporation of a dye in the estimated dosage of scolicide solution injected. A portion of cyst fluid could be withdrawn after 10 minutes exposure to the scolicide and the cyst volume determined colorimetrically by the amount of dilution of the dye. Were the concentration then shown to have been insufficient to kill the scolices, the necessary additional amount of scolicide could be injected.

Summary

Thirty-eight chemicals were compared for their *in vitro* scolicial action upon the hydatid scolices of *Echinococcus granulosus*. Viability of scolices was determined by their motility and staining properties and by their ability to undergo cystic development when inoculated intraperitoneally into young mice. The toxicities of effective scolicides were determined by

their intraperitoneal injection into white mice with the measurement of the LD₅₀ for each. Under the experimental conditions, in combined terms of maximum effective dilution, shortest effective time and least toxicity hydrogen peroxide, Cetrimide, iodine, octoglycine dihydropentiodide, sodium hypochlorite, glycerine, and formalin were the more useful scolicides in the order given.

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